Draft

WORK PLAN

LAHAINA GROUNDWATER TRACER STUDY

LAHAINA, MAUI, HAWAII

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SECTION 1
INTRODUCTION

This Work Plan (WP) was prepared by University of Hawaii (UH) under United States (U.S.) Army Corp of Engineers Cooperative Agreement Number: W912HZ-11-2-0020, for the U. S. Army Engineer Research and Development Center at Vicksburg, Mississippi. It defines the UH scope of services for conducting a tracer dye study from the Lahaina Wastewater Reclamation Facility (LWRF), in Lahaina, Maui, Hawaii. The primary purpose of this WP is to document in detail the procedures that will be used to add a tracer dye to the LWRF effluent stream, monitor for the emergence of the tracer dye at coastal submarine springs sites, and lay out the quality assurance program that will ensure integrity of the tracer study analysis and data. Thus, although important to success of this study, other affiliated aspects of this broad-based study, including airborne thermal infrared imaging and nutrient, major element and stable isotope geochemistry will be described in less detail.

1.1 BACKGROUND

The study area (Figure 1-1) is located on the Hawaiian Islands, in the Kaanapali District of West Maui. The LWRF is about 3 mi north of the town of Lahaina and serves the municipal wastewater need for that community including the major resorts along the coast. The LWRF receives approximately 4 million gallons per day (mgd) of sewage from a collection system serving approximately 40,000 people. The facility produces tertiary wastewaters treated and disinfected with UV radiation to meet R-1 reuse water standards. This water is sold to customers such as Kaanapali Resort to be used for landscape and golf course irrigation. Water not sold is subjected to secondary treatment and chlorinated for disinfection. This water is discharged into the subsurface via four on-site injection wells.

1.1.1 Overview for the LWRF

The LWRF consists of two plants operating in parallel. The first, constructed in 1975, has an average flow capacity of 3.2 mgd, while the other, constructed in 1985, has an average flow capacity of 3.5 mgd (Tetra Tech, 1993). After primary settling to remove a majority of the suspended solids, the LWRF effluent undergoes secondary treatment. This treatment reduces the biodegradable dissolved solids by microbial action that metabolizes the organic matter. A portion of the effluent also undergoes tertiary treatment where this advanced treatment reduces the amount of nutrients, remaining suspended solids, heavy metals, and total dissolved solids. The effluent that is subjected to the tertiary treatment is disinfected using ultraviolet radiation and sold for irrigation use as R-1grade reuse water. R-1 grade reuse water can be used for irrigation with very little restrictions. The treatment for the water not sold as irrigation water is stopped at the secondary level and disinfected by the addition of chlorine. This is classified as R-2 grade water that can be used irrigation, but with restrictions such as no spraying within 500 ft of a property boundary or not being applied to vegetables that are to be eaten raw. In the case of the LWRF, the water is processed to the R-2 grade to reduce the environmental impact of disposal (Limtiaco Consulting Group, 2005).

Limtiaco Consulting Group (2005) summarized the history of the reuse water production and use at the LWRF. Up to the late 1980s, the LWRF provided R-2 water to the Pioneer Mill for sugar cane irrigation. However, with the phase-out of sugar cane this disposal option disappeared. In the mid-1990s Maui County upgraded the plant to produce R-1 water to address concerns about seasonal algae blooms. This water is sold primarily to the Kaanapali Resort and golf course. Other uses include roadside landscape irrigation and dust control. The distribution system was extended to make R-1water available to the Maui Land and Pineapple Company for pineapple irrigation in 2003. This water was to be blended with non-potable water from the Honolua Ditch. However, due to ample rain and the phase-out of pineapple, little use has been made of this option. This infrastructure may be beneficial to the emerging diversified agriculture on west Maui.

The LWRF injects the chlorinated R-2 grade effluent into four injection wells (Figure 1.2, 1.3). Under the Safe Drinking Water Act an Underground Injection Control (UIC) permit is required from the U.S. Environmental Protection Agency (USEPA) for the injection of subsurface wastewater effluents that might affect potential sources of drinking water. The LWRFs UIC permit expired on June 6, 2005 but the facility is operating under the expired permit until a renewal is approved. Sections 1421 through 1445 and Section 1450 of the Safe Drinking Water Act require that each state establishes an UIC program to protect drinking water sources from contamination due to sub-surface fluid injection. Title 40 of the Code of Federal Regulations, Parts 144 through 148 details the UIC permit regulations. Part 144 lays out the minimum permitting and program requirements. Part 145 details the elements and permitting procedures for a state program, while Part 146 spells out the technical requirements. Part 147 sets forth the UIC program for each state including Hawaii. Much of the oversight of UIC activities is delegated to the states. In Hawaii, the UIC program requirements are codified in the Hawaii Revised Statutes (HAR) Title 11, Chapters 23 and 23a.

The UIC restrictions are less stringent if an aquifer is not a potential source of drinking water due to high concentrations of total dissolved solids (TDS). This applies to the area of aquifers that are seaward of an UIC Line and are classified as exempted aquifers. Class V injection wells are allowed in exempted aquifers and this class includes the injection of sewage derived wastewater. The LWRF is located seaward of the UIC line (Figure 1-2) and injects treated effluent to depths of between -55 and -229 feet above mean sea level (ft msl). The screen length or open interval of the wells varies from 95 to 150 feet (ft). Table 1-1 gives the construction details for the injection wells. The average summer injection rate is approximately 4.0 mgd. The daily maximum rate is 19.8 mgd and the maximum weekly average injection was 9.0 mgd (County of Maui, 2010). As mentioned earlier, Maui County is in the process of renewing the UIC permit for these wells. However, concerns about the impact of injection well operations have on the coastal environment has prompted research into the amount, distribution, and discharge points of nutrients and other chemicals into the marine environment.

Table 1-1. Construction Details of the LWRF Injection Wells

Injection Well No.	**1**	**2**	**3**	**4**
Construction Date	1979	1979	1985	1985
Elevation (ft msl)	33	33	28	29
Total Depth of Well (ft bgs)	200	180	225	225
Solid Casing Length (ft)	88	88	108	108
Bottom of Well (ft msl)	-168	-150	-200	-229
Screen/open hole length (ft)	115	95	120	150
Top of Screen/Open Hole elevation (ft msl)	-55	-55	-80	-79
Bottom of Screen/Open Hole Elevation (ft msl)	-170	-150	-200	-229

Scientific evidence (Dailer et al, 2010; and Hunt and Rosa, 2009) strongly supports the hypothesis that effluent injectate from the LWRF is discharging into the nearshore waters southwest of the plant. However, to date, the extent of that link has not been irrefutably established. One of the goals of this project will be to tag the effluent with a fluorescent dye prior to injection and monitor the near shore for emergence of the dye at nearby coastal submarine springs, particularly those identified by Hunt and Rosa, 2009 and Dailer et al, 2010). Figure 1-3 shows location of the submarine spring relative to the LWRF.

1.2 PROJECT SUMMARY

The purpose of this study is to provide critical data about the hydrological connection between the injected effluent and the nearby coastal waters, confirm the locations of emerging injected effluent discharge in these coastal waters and determine a travel time from the LWRF injection wells to the coastal waters. This will provide a firm basis for estimating the total flux of effluent to the near shore environment. Data gathered in this study will be incorporated into a groundwater flow and transport model. The transport module of the model will be capable of simulating sorption and tracer degradation.
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1.3 HISTORY OF RELATED INVESTIGATIONS

Examples of relevant studies include those related tracer testes on the islands, including Maui (e.g. Tetra Tech, 1994), and those concerning the potential linkages between effluents and algae blooms (e.g. Dollar and Andrews, 1997; Borke, 1996). More recent scientific investigations on Maui include Hunt and Rosa's (2009) multi-tracer approach to detect effluent discharges in Lahaina and Kihei, Dailer et al.'s

(2010) extensive work using stable isotope data from intertidal and near shore cultivated algae, and recent groundwater investigations for West Maui modeling by the USGS (Gingerich, 2008).

In response to concerns prompted by seasonal algae blooms in West Maui, the USEPA sponsored a nutrient balance study of West Maui (Tetra Tech, 1993). This report identified the LWRF as one of the three primary nutrient release sources to Lahaina District coastal waters, with sugar cane and pineapple cultivation being the other two. This study ranked the LWRF second in annual nitrogen contribution to the Lahaina District coastal waters and first in phosphorous contribution. Since this study was completed, the cultivation of both sugar cane and pineapple has been sharply curtailed. This implies that the LWRF is the primary contributor of nutrients to water in the study area. The Tetra Tech study also estimated the travel time of effluent from the point of injection to the coast using a two dimensional numerical flow model. Based on that model, the travel time could be as short as 10 days. In absence of any injection, the travel time would be increased to 50 days based on the average groundwater-flow velocity. The model assumed an aquifer thickness of 20 ft. Using the Ghyben-Hertzberg principle, the freshwater lens is 41 times the groundwater elevation above sea level (Fetter, 1988), which yields a more accurate aquifer thickness of 80 to 100 ft near the LWRF. This is based on a water table elevation of 2 to 2.5 ft msl (Gingerich, 2008). The thinner modeled aquifer thickness would result in a shorter travel time. Also, the distance between the LWRF injections wells and the nearest identified submarine spring is approximately 0.49 mi, which is greater than the direct path distance to the shoreline. The eastern boundary of the Tetra Tech model was the interface between the high level water at the interior of the island and the basal groundwater. This was assigned as a no-flow boundary condition. In actuality, however, there is significant groundwater flow from the high-level water body to the basal groundwater (Gingerich, 2008).

Since the LWRF was identified as a major contributor of nutrients to the marine environment in the 1993 study, an effluent fate and transport study was commissioned by the USEPA. Tetra Tech (1994) conducted a tracer test to identify the submarine locations where the effluent was discharging into the marine environment. They added Rhodamine WT (RWT), a fluorescent tracer dye, into the effluent stream prior to underground injection at a concentration of approximately 100 parts per billion (ppb). This injection last for 58 days. To monitor for the emergence of the effluent tagged with RWT, they completed a series of monitoring transects offshore north-northeast transects. Every 200 yards, a pump suction was let drift to the ocean bottom. The suction line was connected to a pump on the survey boat with the discharge from the pump ported through a constant monitoring fluorometer. In that study, only two occurrences of elevated fluorescence were detected at one sampling location, in the southwest corner of their sampling grid (Figure 1-4). The fluorescence value was low, about three times that of background. The first detection occurred 55 days after the start of injection and the second detection occurred 61 days after the start of injection. The location of the Tetra Tech elevated fluorescence detections was very near the submarine springs identified by Hunt and Rosa (2009) and Dailer et al. (2010) as probable discharge points for the LWRF effluent. Due to the fluorescence values being only slightly above background, it is uncertain whether the source was the Rhodamine WT dye, or another fluorophore such as dissolved organic matter. Figure 1-4 illustrates the location where Tetra Tech detected RWT fluorescence, the submarine springs suspected of discharging effluent, and the plume area proposed by Hunt and Rosa (2009).

Hunt and Rosa (2009) investigated the use of multiple in-situ tracers to investigate where and how municipal wastewater effluent discharges to the nearshore marine environment. They investigated the nearshore marine waters adjacent to effluent injection sites in Lahaina and Kihei, Maui. They concluded that the most conclusive tracers were the presence of pharmaceuticals, higher proportion of the heavy nitrogen-15 (^{15}N) isotope compared to the more abundant nitrogen-14 (^{14}N) isotope in nitrate and in coastal benthic macroalgae, and organic waste indicator compounds. Particularly pertinent to this Work Plan, they investigated background fluorescence along the shoreline near the LWRF, where they measured fluorescence with a handheld fluorometer with an optical brightener and a Rhodamine WT channel. They detected optical brightener fluorescence in samples collected at the submarine springs that was 15 times that in the water column near the springs. There was no difference in Rhodamine WT fluorescence between the submarine spring and the water column samples. This indicates that non-dye fluorophores in LWRF effluent were probably not responsible for the elevated Rhodamine WT fluorescence detected by Tetra Tech. This further indicates that the elevated fluorescence in the RWT wavelength detected by Tetra Tech was likely from the dye they added to the effluent.

Dailer et al. (2010) used the ratio of ^{15}N:^{14}N ($\delta^{15}N$) in algae to map the anthropogenic input of nitrogen to the nearshore waters of Maui. Atmospheric and fertilizer $\delta^{15}N$ fall in the range of -4 to 4 parts per thousands ($^{o}/_{oo}$). Input from sewage can be identified by its higher $\delta^{15}N$ values that range from 7 $^{o}/_{oo}$ to 38 $^{o}/_{oo}$ (Dailer et al., 2010; Kendall, 1998; and Gartner et al., 2002). The two highest $\delta^{15}N$ values (33.2 and 43.3 $^{o}/_{oo}$) were found at two sites near the LWRF. In the Lahaina area, they also observed submarine springs that were warmer than ambient seawater surrounded by a black precipitate thought to be iron oxides.

Significant work has been done on the wastewater injection and the fate of this injectate in Hawaii. Oberdorfer and Peterson (Oberdorfer and Peterson, 1982; and Oberdorfer, 1983) studied the processes that lead to injection well clogging and the fate of nutrients in the injected effluent. They found that a significant amount of denitrification occurs in the subsurface after injection. Petty and Peterson (1979) investigated sewage injection practices in West Maui including resorts and condominiums. The fate of wastewater injection plumes has been modeled by Hunt (2007), Burnham et al., (1977), Wheatcraft et al. (1976), and Tetra Tech (1993). All showed that once the wastewater effluent is injected, the plume tends to rise due to its positive buoyancy.

There have been several chemistry surveys and studies of anthropogenic inputs into the coastal waters of West Maui in addition to those already cited. Laws et al., (2004) showed that coastal nutrient concentrations exceeded State water-quality standards for marine waters. Street et al. (2008), investigated submarine groundwater discharge (SGD) using multiple tracers such as radon/radium pair, silica, and salinity. They estimated that the SGD near the study site was 0.07 to 0.12 meters cubed (m^3) per meters squared (m^2) per day (d), delivering a dissolved inorganic nitrogen load of 13.3 to 36.8 millimoles per m^2/d. Dollar et al. (1999) and Atkinson et al. (2003) monitored for estrogen as indicator of discharge of cesspool effluent to the waters of west and south-central Maui. Soicher and Peterson (1997) studied the nutrient input to West Maui coastal waters and concluded that stream discharges were an acute nitrogen source, but chronic SGD was the major contributor.

1.4 DESCRIPTION OF CURRENT STUDY

1.4.1 Project Objectives and Scope

The purpose of this proposal is to provide critical data about the hydrological connection between the effluent discharge and the coastal waters, confirm the locations of emerging discharge of injected effluent into the coastal waters, and determine a travel time from the Lahaina Wastewater Reclamation Facility's (LWRF) injection wells to the coastal waters. The purpose of the tracer test is to investigate any linkage that may exist between the underground injection of treated municipal sewage effluent into the sub-surface waters near the town of Lahaina, Maui, Hawaii, and the discharge of that effluent to near shore waters close to the treatment facility. If a linkage is found, the study will estimate the time of travel from the LWRF to the submarine springs. The study will also estimate the total flux of effluent being discharged into the near shore waters. The primary approach for this study will be the addition of fluorescent dye(s) to the effluent prior to injection followed by a robust surveillance program at the near shore waters to monitor any dye that reaches the near shore marine environment. The work will be supplemented by two detailed isotope and geochemistry surveys. We will also complete a survey of radon, radium and current meter measurements at the submarine springs. These will be done prior to, and following the dye addition to provide two chemical reference points to aid in estimating the effluent flux, its variability, and the effluent time of travel. This study will also evaluate the amount of mixing between the effluent and the native water prior to being discharged into the near shore environment. Groundwater and transport modeling will be used to interpret the tracer breakthrough curve. The tracer breakthrough curve is the bell-shaped graph of tracer concentration at the downgradient sampling point versus the time since dye addition. From this curve, time of travel and the degree of dispersion can be estimated.

1.4.2 Project Tasks

The following tasks will be performed during this study.

1. A review of scientific studies, reports, maps, and other sources will be performed to gain the necessary knowledge to conduct this study.

2. A field reconnaissance will be conducted of the study area. This will include visiting the LWRF to become familiar with the site and its operations. Our study will also conduct an aerial infrared survey of the coastal waters of West Maui to map sea surface temperature anomalies. These anomalies are indicative of submarine groundwater discharge. This data will be reviewed to locate any unidentified effluent discharge areas, as well as aid in determining the extent of SGD waters derived from the LWRF versus those emanating as native SGD.

3. A background characterization of the study will be performed to document the fluorescence of the suspected effluent discharge areas and to map the variability of their chemistry. This will include sampling the suspected effluent discharge sites to determine correction factors that will be incorporated into the dye tracer analysis program. Marine, LWRF effluent, and groundwater samples will be collected and analyzed for nutrient content, major ion composition, and carbon,

nitrogen, oxygen, radon and radium isotope composition. This will allow us to approximate the partitioning of the submarine spring discharge water between groundwater, effluent, and recirculated seawater sources.

4. A tracer study will be performed to tag the LWFR effluent with tracer dye and monitoring for the emergence of the dye at nearshore submarine springs.

5. The results of the previous tasks will be combined and evaluated to assess the degree of hydraulic connection between the LWRF and the near shore submarine springs.

6. A comprehensive report will be written to document the activities, data collection, and the conclusions of this study. This report will evaluate the degree of hydraulic connectivity between the LWRF and the submarine springs, and the magnitude of nutrient contribution from this facility to the nearshore environment.

1.5 PROJECT ORGANIZATION

1.5.1 U.S. Army Corp of Engineers Program Manager

Ms. Cindy S. Barger, Watershed Program Manager, U.S. Army Corp of Engineers

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1.5.2 Hawaii Department of Health Program Manager

Mr. Daniel Chang is the Hawaii Department of Health (HDOH) program manager for this study.

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1.5.3 University of Hawaii Principal Investigators

Dr. Craig Glenn – is an expert in submarine groundwater discharge investigations using multiple techniques such as stable isotope geochemistry, seep flow measurement, and TIR. He is the team leader, and will supervise the geochemical and the TIR investigations. Dr. Glenn is the Lead Principal Investigator and will be the primary UH point of contact for project and ensure the other PIs and UH personnel.

Phone: (808) 956-2200
Email: glenn@soest.hawaii.edu

Dr. Aly El-Kadi – is an engineer and hydrogeology professor, and researcher with expertise on numerical groundwater and transport modeling. He will be supervising the tracer test design, implementation, and the numerical modeling.

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Email: elkadi@hawaii.edu

Dr. Henrieta Dulaiova – has extensive experience studying submarine groundwater discharge in various geological settings. She uses stable and radio isotope tracers and direct methods to study spatial distribution and temporal variability of SGD. She will be supervising the radon and radium data collection and analysis, current meter deployment, as well as aiding in the geochemical interpretations.

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Meghan Dailer – is a marine biologist that specializes in stable isotope interpretations of marine plants. She has over four years of experience working at the study site and will supervise marine field operations.

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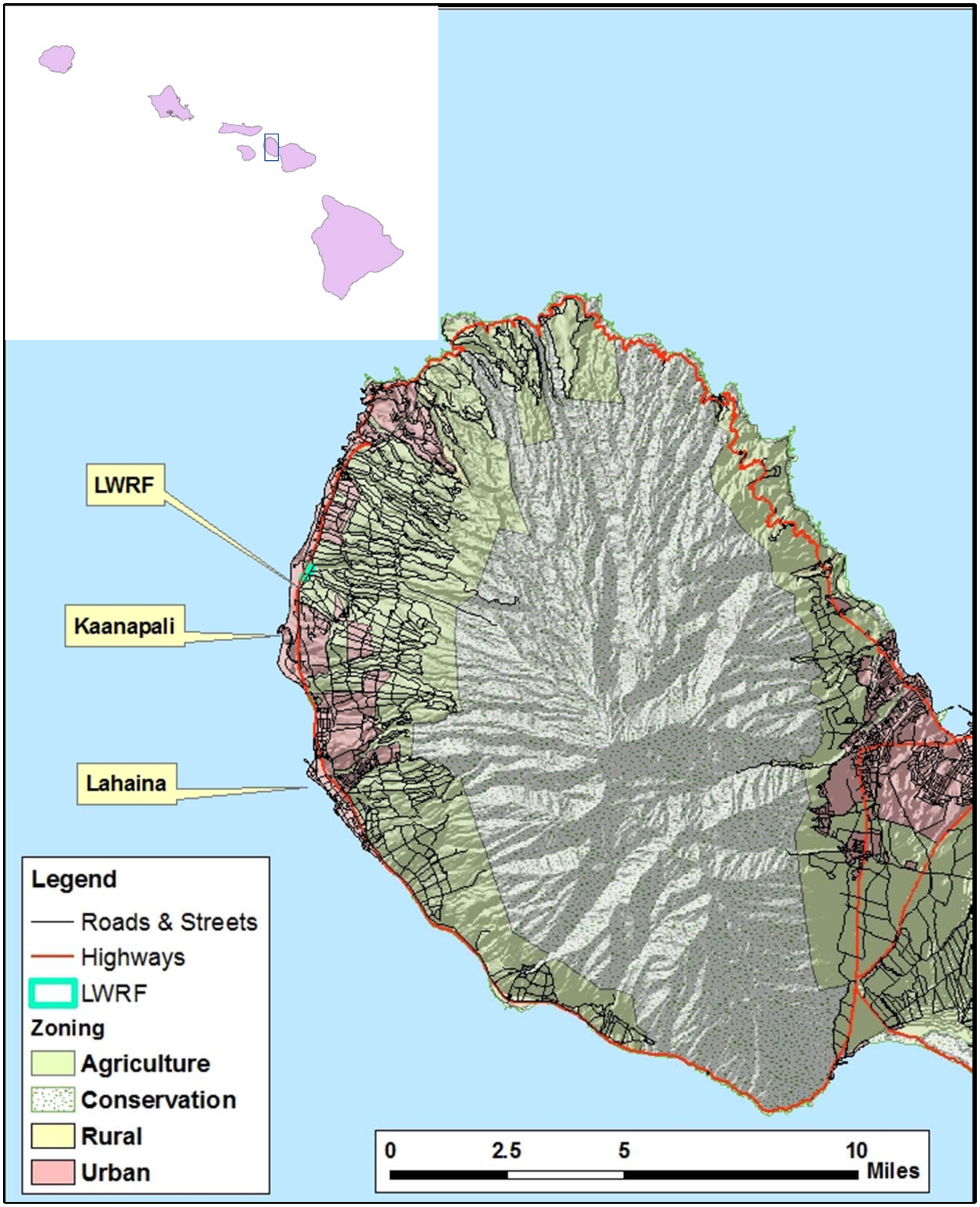

Figure 1-1. Map Showing the Location of the LWRF Study Area

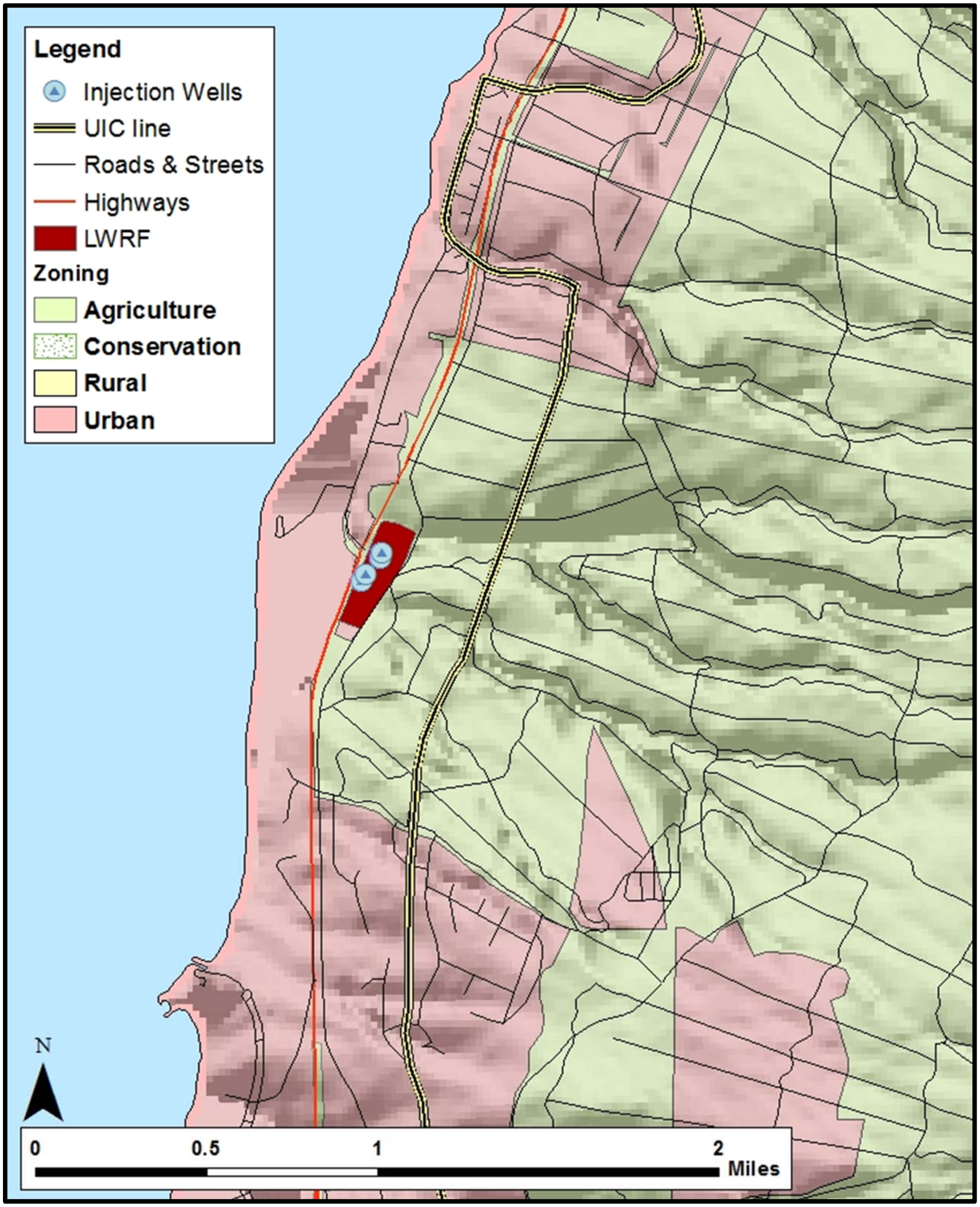

Figure 1-2. The Location of the LWRF in Relation to the Coast and the UIC Line

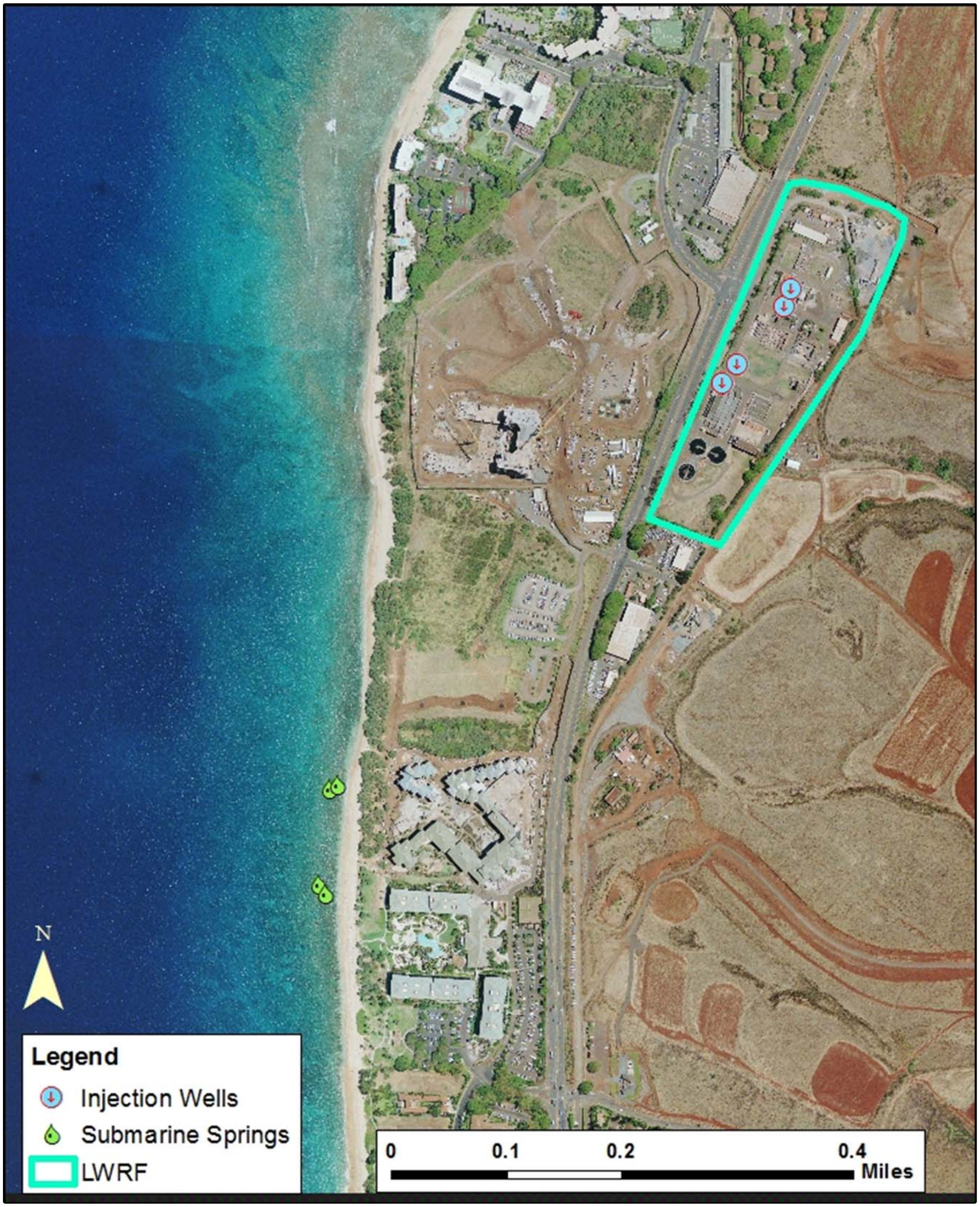

Figure 1-3. Map of the LWRF, Injection Wells, and Submarine Springs

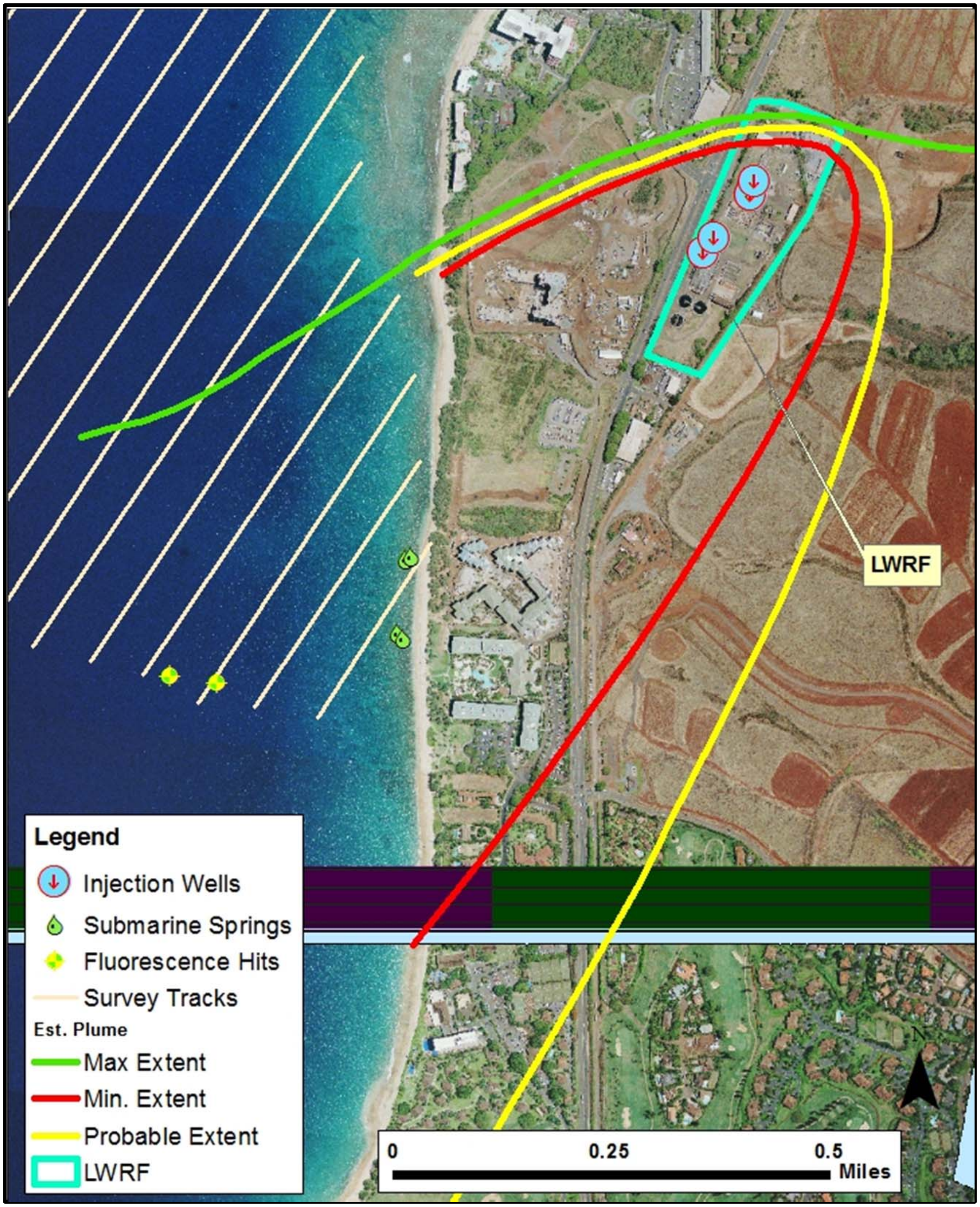

Figure 1-4. Comparison of the Location of the Submarine Springs (Hunt and Rosa, 2009; Dailer eta al., 2010), Possible Plume Extent as Proposed by Hunt and Rosa (2009), and Offshore Sample tracts and Two Occurrences of Elevated Fluorescence ("Hits") Measured by Tetra Tech (1994).

SECTION 2
SITE DESCRIPTION AND BACKGROUND INFORMATION

Located between 155° 57' and 156° 42' west longitude, and 20° 34' and 20° 59' north latitude, Maui lies near the middle of the Pacific Ocean far from any continental land mass. Maui is part of an island chain that is formed as the Pacific Tectonic Plate passes over a mid-ocean hotspot. The primary shield volcanoes forming this island chain generally occur in parallel trending pairs (Langenheim and Clague, 1987). Maui is no exception to this trend, consisting of the East Maui Volcano, Haleakala, and the West Maui Volcano. The isthmus between the West Maui Volcano and Haleakala provides a natural division between eastern and western Maui.

2.1 LWRF Environmental Setting

2.1.1 Geology

The site of this study is located on the northwestern extent of the West Maui Volcano. This is the older of the two Maui shield volcanoes. Figure 2-1 shows the geology of West Maui (Sherrod et al., 2007). This volcano differs from most Hawaii Shield volcanoes, in that, instead of having well defined linear rift zones, the dikes occur in a radial pattern emanating for a central caldera. Being the older of the Maui shield volcanoes, the West Maui Volcano is more heavily eroded resulting in a well-developed radial stream system. The West Maui volcano is made up of two major volcanic units, the Wailuku Basalts and the Honolua Volcanics. The Lahaina Volcanics, a rejuvenation stage eruption, are a minor unit of the West Maui Volcano. The Wailuku Basalts make up the vast bulk of the West Maui Volcano. These lavas were erupted during the shield building stage of the West Maui Volcano. They are generally thin bedded theoliitic basalts, ranging in thickness from 1 to 100 ft (Stearns and McDonald, 1942; Gingerich, 2008). Isotopic dating places the origin of these lavas in the Pleistocene age (1.8 million to 11.5 thousand years ago). The thin bedded nature and the high incidence of interflow boundaries, makes the hydraulic conductivity of these lavas very high. The Honolua Volcanics were produced by late eruptions. These lavas are more massive and tend toward andesitic composition. Due to the increased thickness and denser nature, the hydraulic conductivity of the lavas is much lower than those of the Wailuku basalts. The Honolua Volcanics overly the Wailuku basalts and generally only intersect the water table nearer to the coast. They are more prevalent on the northeast and northwest slopes of the West Maui Volcano (Gingerich, 2008; Sherrod et al., 2007) and do not intersect the water table in the study area. The lower permeability these lavas and the higher recharge in the upper elevations can also support perched waters (Stearns and Macdonald, 1942).

The Lahaina Volcanics resulted from rejuvenation stage eruptions that took place 610,000 - 385,000 years ago. As with the Honolua Volcanics they are more massive in nature. However, their small areal extent and proximity to the coast makes this unit less important when assessing groundwater flow than the other volcanic units. A vent that produces Lahaina Volcanics is located in the southwest portion of the study area (Refer to Figure 3-2).

2.1.2 Regional Groundwater Hydrology

The precipitation that falls on West Maui is partitioned between surface runoff, evapotranspiration, soil moisture storage, and groundwater recharge. Recharge, (the fraction of groundwater that reaches the water table), flows radially out from the central highlands to discharge areas along the coast. Figure 2-2 shows the recharge distribution for West Maui and the extent of the high level water body (Engott and Vana, 2007; and Gingerich, 2008). Recharge ranges from 350 inches per year (in/yr) at the high elevations to less than 10 in/yr along the coast. The high recharge and low hydraulic conductivity of the dike zones in the interior regions of the West Maui Volcano result in a water table with elevations up to 3,000 feet above mean sea level (ft msl) (Gingerich, 2008). Figure 2-1 shows the approximate interface between the high level and basal aquifers (Mink and Lau, 1990). The dike impoundment of the groundwater is breached in areas where erosion has cut deep valleys and subterranean water provides baseflow for the streams. Due to the radial nature of the dike zone for this volcano, the high level water body forms an oval rather than the linear aquifers of this type found on most Hawaii volcanoes.

In the subsurface, once the groundwater flows out of the high level water body, it becomes a lens of freshwater floating the underlying saltwater with a water table elevation of less than a few tens of feet above sea level. This Ghyben-Herzberg principal that states the thickness of the freshwater lens is 41 times the elevation of the water table above sea level is only an estimation, however, and the actual thickness of the freshwater lens can deviate from this value due to factors such as non-horizontal flow and heterogeneous geology (Izuka and Gingerich, 1998). The mixing of the two waters in the basal lens along the groundwater flow path results in a sloping transition rather than a sharp interface between fresh and saltwater.

As the groundwater approaches the shoreline, it may encounter sedimentary deposits and formations that retard its flow. These formations, referred to collectively as caprock, have an effective hydraulic conductivity that is significantly lower than that of thin bedded lavas. This results in a thicker freshwater lens due to the higher water table and a barrier that retards saltwater intrusion into the aquifer. Drilling logs from the injection wells at the LWRF indicate that sedimentary deposits extend below the water table resulting in an overlying confining layer for that portion of the aquifer between the facility and the coast (Maui Co., 2004). Preferential flow paths in the aquifer can result in well-defined submarine springs, as seems to be case in this study area. A more diffuse discharge may be present in addition to these preferential flow paths.

The water transport characteristics of the various aquifer materials vary greatly along the flow path. The hydraulic conductivity of the dike-intruded lavas in Hawaii is estimated to range from 1 to 500 ft/d (Hunt, 1996). The low end of this estimate would be more representative of the West Maui Volcano due to the high density of dikes in the high water body. In a groundwater model of West Maui, Gingerich (2008) assigned a horizontal hydraulic conductivity of 2,097 ft/d and a vertical hydraulic conductivity of 10.5 ft/d for the Wailuku Basalts in the Lahaina area. For the sedimentary deposits he used values of 17 and 0.38 ft/d for the horizontal and vertical hydraulic conductivity, respectively.

2.1.3 Land Use

In West Maui, the land utilization is divided between an urban center in the Lahaina area, pineapple cultivation on the western alluvial terrace of the West Maui Mountains, and resort development along the western coastal region. Most of the pineapple and sugar cultivation land has ceased. Portions of this land remain fallow or have been converted to low density housing and diversified agriculture. Much of the interior of the West Maui Mountain is undeveloped and zoned as conservation land. Figure 2-3 shows the current land use zoning for West Maui (Hawaii Office of Planning, 2010).

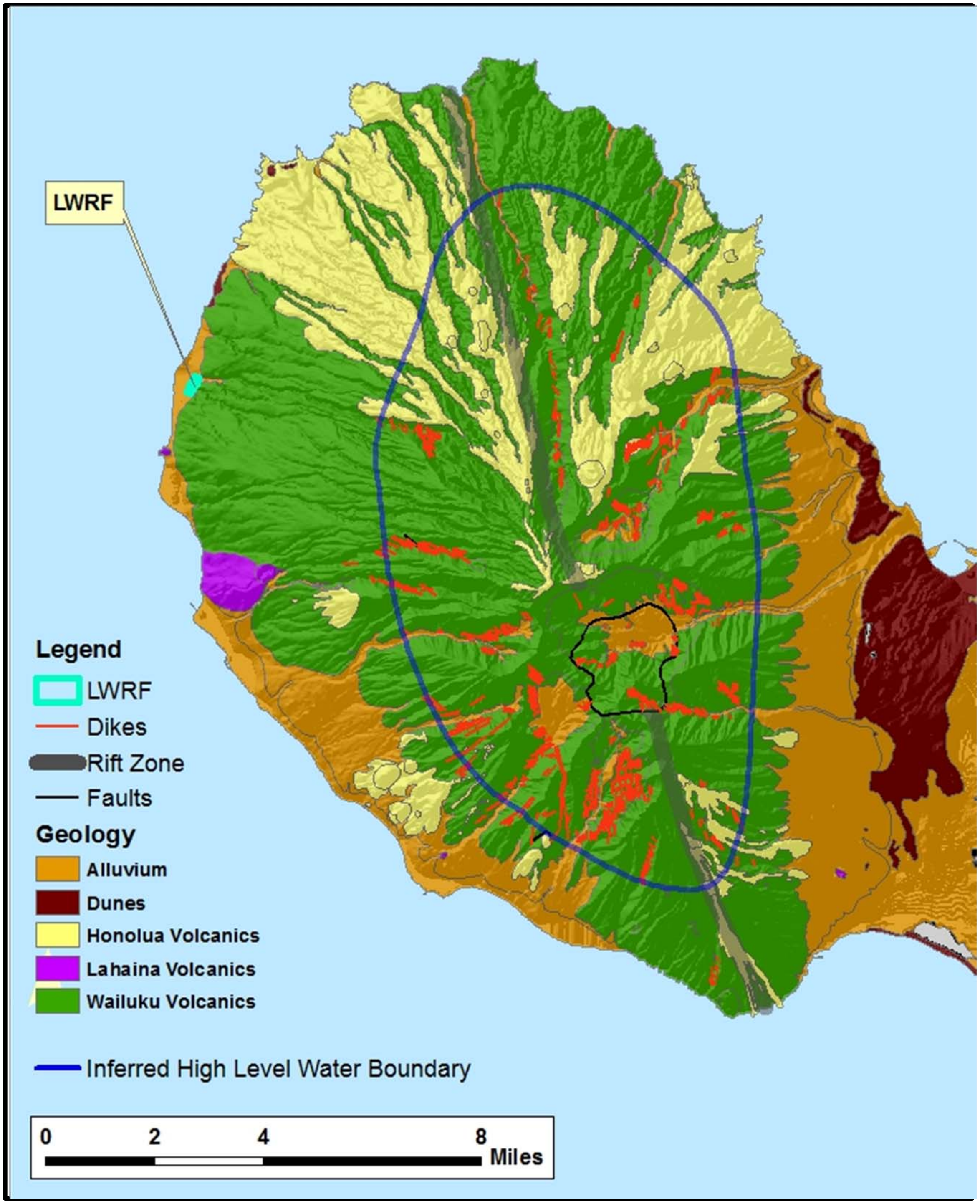

Figure 2-1. West Maui Geology (Sherrod et al., 2007) and the Inferred Boundary Between the Central High Level Water and the Peripheral Basal Groundwater Lens

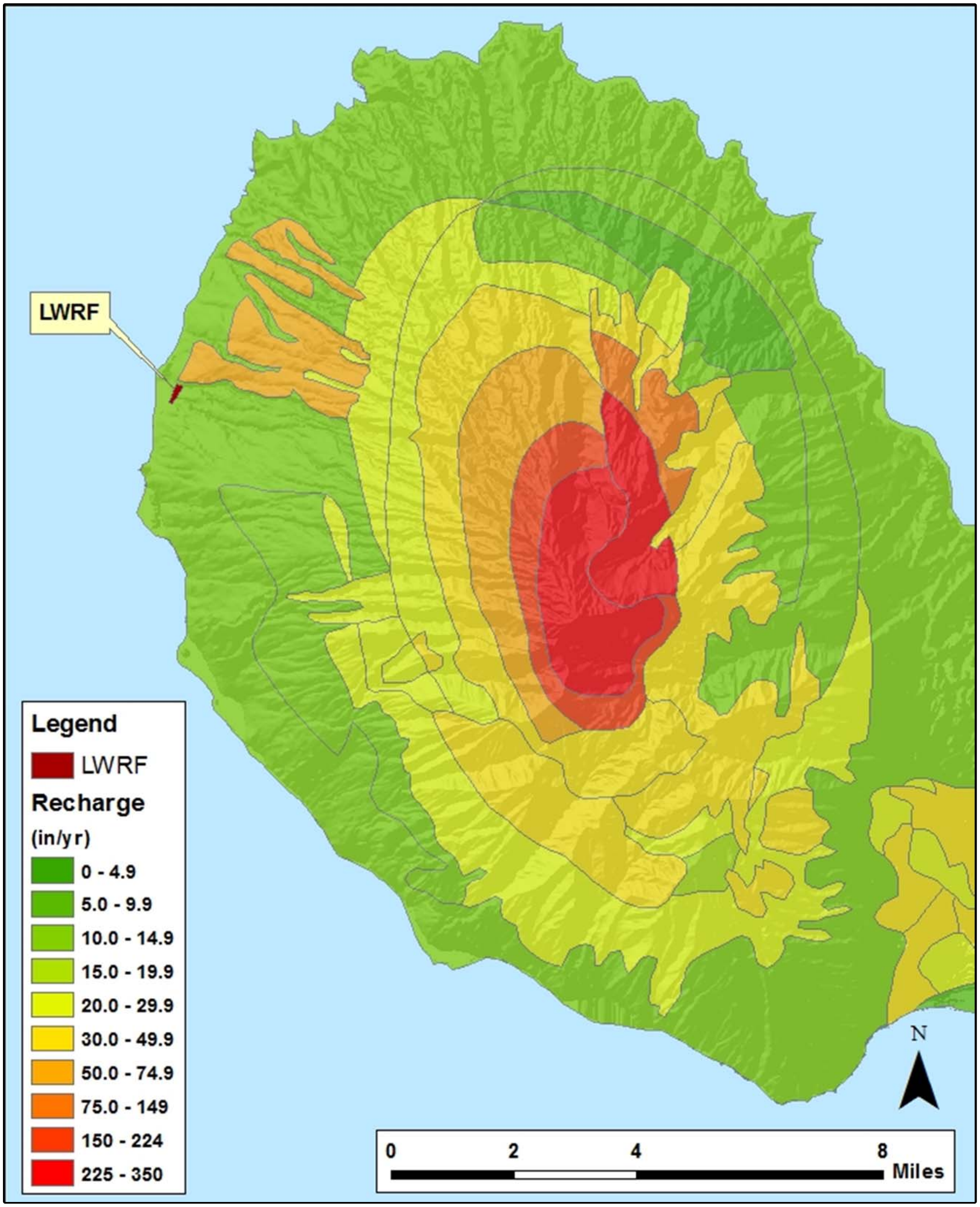

Figure 2-2. Groundwater Recharge Distribution in West Maui (Engott and Vana, 2007; and Gingerich, 2008)

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SECTION 3 STUDY DESIGN

3.1 SITE CHARACTERIZATION AND SURVEY OF BACKGROUND CONDITIONS

3.1.1 Site Reconnaissance

The following field mobilization activities will be conducted:

- Visit the LWRF,
- Investigate available wells,
- Survey coast for submarine springs, and
- Meet with local stakeholders

3.1.2 Airborne Thermal Infrared Survey

High-resolution, georeferenced, black body and in situ surface ocean temperature corrected, airborne thermal infrared (TIR) sea surface temperature mapping will be used to study the submarine groundwater discharge (SGD) to area in order to ascertain an accurate depiction of the ocean input locations, the overall aerial extent and distribution, and seaward oceanic mixing of water from terrestrial and ocean water (e.g. Johnson et al., 2008). These maps will also form the necessary and fundamental base maps for coordinating the land and ocean based activates of the project.

3.1.2.1 TIR Utilization for the Tracer Test Study

The TIR survey method will be used because there are temperature and density differences between coastal marine waters and discharging terrestrial groundwater and effluents. Injected effluent is warmer than naturally occurring groundwater, and the submarine springs at Kahekili Beach near Lahaina are notably described as "warm seeps." Hunt and Rosa (2009) demonstrated that the temperatures near the Kahekili Beach springs were elevated, and their work showed that at the Kihei injection site the effluent (27.5°C) was 7°C warmer than regional groundwater (20.5°C at an up gradient well). These temperature differences between native SGD, effluent-bearing SGD, and seawater are clearly resolvable with our technique. Because the colder SGD and warm effluent are less saline than the coastal seawater, it is extremely likely that both produce buoyant surface water plumes that originate from seepage from the seafloor and along the beach face at a wide variety of scales, as well as more diffuse discharges, all of which may be differentiated and mapped within the study area using the TIR methodology.

The geo-referenced aerial TIR base map (resolution: 0.5 $^{o}C/m^2$ pixel) of the area's seawater surface will be produced at the onset of the study to precisely delineate between emergence areas of cold groundwater and those of warmer effluent waters. Our time estimate for internal use of the TIR maps is 1-2 weeks for preliminary processing for immediate use, and 2-3 weeks for final ground-truth temperature corrected digital maps.

The flight survey will be conducted at an altitude of 7000 feet using a TIR spectrometer (sensitivity of 0.1°C) mounted to an aircraft's fuselage. Figure 3-1 shows the planned flight paths. To eliminate solar heating and minimize land-sea thermal contrast, TIR flight accusation will take place overnight between approximately midnight (departing from Oahu) and 5:30 a.m. (returning to Oahu). After the aerial surveys, all flight line images will be spectrally calibrated to blackbody measurements acquired during the flight. All spectral bands between 8.1 and 11.0 nm in the calibrated images will be averaged to a single band and subsequently converted to temperature by inverting the Planck Equation. The temperature-converted imagery will be georeferenced using the aircraft's navigational parameters, referenced to regional aerial photographs using tie points, and then mosaicked to form high-resolution (3.2 square meter pixels) surface water temperature maps. The temperatures in the mosaicked images will then be calibrated to field emplaced *in situ* thermistors (accuracy = 0.5°C; field plan described below) to correct for atmospheric interferences (e.g. water vapor and aerosols) between the ocean's surface and the airborne sensor.

3.1.2.2 TIR Ground Truthing

This initial phase of fieldwork will occur in close coordination with the TIR flight and is critical for making accurate corrections between the TIR flight data and *in situ* surface water temperatures. During this operation, a network array of approximately 10 *in-situ* self-recording thermistors will be deployed throughout the study area during the day prior to the night of the TIR flight accusation, and retrieved during the day immediately following. The field team for this operation will also be in direct cellular contact to satellite weather interpretation via the NOAA Weather Service Office personnel in Honolulu (housed at the Hawaii Institute of Geophysics Building, SOEST), and will work through the night to advise the flight operation crew of this data and their own ground-observed conditions of cloud cover via cellular and radio.

3.1.3 Environmental Sampling

3.1.3.1 Sample Piezometer Installation

Samples from the submarine springs will be collected using piezometers (Solinst Model 615 6" Drive-point piezometers) deployed directly in the cracks where the water discharges. The piezometers will be inserted using a slide hammer with the help of divers. Polyethylene tubing will be attached to the piezometers and a peristaltic pump will be used to draw water to a boat. This installation will be used to sample water from the submarine springs for radon, nutrients, stable isotopes and radium isotopes as well as later on of the injected tracer. Depending on effectiveness and sample recovery experienced in the field, smaller-diameter piezometers may also be deployed for extracting water samples from the beach face.

3.1.3.2 Radon/Radium Survey

Naturally-occurring radioisotopes of radon and radium will be surveyed and monitored to ground-truth and further define the locations of SGD submarine springs revealed by TIR Aerial Survey, as well as at the seep sites previously identified in past studies (e.g. Hunt and Rosa, 2009; Dailer et al., 2010). Radon and radium are released from rocks and sediments within the aquifer and groundwater flowing through these gets highly enriched in these isotopes. Ocean water contains negligible concentrations and this high concentration gradient makes these isotopes excellent tracers of subterranean groundwater discharge. We will measure these isotopes along the coastal zone encompassing at least 3.1 mi of the coastline to also include areas adjacent to the location where the plume is thought to be discharging, and with these measurements we will identify and differentiate the source(s) of discharging water (fractions of fresh groundwater, injectate, and recirculated seawater). We will complete continuous coastal surveys with an autonomous radon sensor, which will be used for both the spatial survey (mapping) and time-series monitoring. For the spatial survey, we will employ two of these instruments at the same time, one looking at the surface plumes and one at benthic inputs. In addition, radium samples will be collected and analyzed. From the chemical mass balance and isotope signatures of the individual water masses we will thus be able to assess each localized groundwater discharge flux with special focus on the locations where the injectate plume(s) are thought to be discharging, and also including a wider area to identify any additional discharge sites that have not been previously considered or were not found because they are too diffuse or far offshore. We will also be able to quantify the variability of the specific background and effluent discharge rates over the time duration of one week prior and one week following dye injections, as well as be able to uniquely position ourselves for integration of these results into the longer-term monitoring efforts planned.

3.1.3.3 Stable Isotope and Geochemical Sampling

The partitioning of seep discharge water described above requires a comprehensive characterization of the identified end-members. Our sampling plan will include both nutrient and geochemical sampling of the LWRF effluent, and groundwater upgradient and cross-gradient from the LWRF. These data are critical for portioning the submarine seep fractions between the possible sources and to estimate the nutrient load contributed by the LWRF.

To estimate fluxes of injected wastewater into the ocean at different groundwater portals, the volumetric fraction of wastewater in the groundwater will be multiplied by the groundwater velocity measured using a current meter. Groundwater discharged to the ocean from the Lahaina coastline is the final chemical mixtures of several chemically distinct starting “source water end members,” particularly (1) LWRF injectate, (2) remaining nutrients from past agricultural practices, (3) soil water, and (4) recirculated subterranean ocean water. The goal of the geochemical modeling portion of the work, therefore, is to quantify the portion of each of these source water end-members at coastal discharge sites. Once each site is characterized, those where the LWRF injectate fraction is greatest will be given priority for sampling during the subsequent phase of this study. Carbon isotopes will be used to distinguish the extent of bacterial degradation (suboxic to anoxic diagenesis) occurring within the aquifer, as well as help distinguish between dominant inputs via soil waters, anthropogenic activity, and rainfall/ocean water sources in groundwater. Nitrogen isotopes of the dissolved nitrate in the waters will be utilized to identify natural and anthropogenic sources of nitrate (commercial fertilizers vs. organic soil nitrate vs. human waste) (e.g. Kendall and McDonnell, 1998, and papers therein). $\delta^{18}O + \delta^{15}N$ pairs of dissolved nitrate allow further potential delineation between atmospheric nitrate, fertilizer nitrate, and soil nitrate. We will also deploy loggers that measure conductivity, depth and temperature directly next to several submarine springs to record any changes of the seep discharge salinity attributable to the changes in the volume of the injected effluent. These flux estimates will then be compared to those derived from the radon/radium isotope systematics described above to validate and refine where the maximum discharge estimates of all the injectate occurs so as to best position ourselves for the coastal portions of the tracer addition and monitoring phase of the project.

3.1.3.4 Submarine Spring Discharge Characterization

Data collected during the radon mapping/monitoring and multi-geochemical sampling will be combined with direct discharge measurements at submarine springs, to quantify the specific flow velocity and lateral dispersion of the freshwater discharge and its component effluent to the near shore environment. The seep discharge rates will be measured directly with a YSI AquaDopp Profiler® (an Acoustic Doppler Current Profiler, or ADCP). This instrument, as equipped with a 1 megahertz right angle head, will be used to profile current velocities in three dimensions over distances as short as 1 meter, or as long as 100 meters. We will deploy the ADCP in the close proximity mode to measure a three dimensional profile of the current discharging from each major seep we identify. When combined with chemical partitioning results described above, the ADCP measured profiles will also further quantify the volume and specific flow rate of natural groundwater and possible effluent contributions discharging from each seep. A rudimentary time of travel from an injection point to a coastal discharge point can also be estimated from this data, which will greatly aid us in refining our calculations for rates of tracer dye additions and durations during Phase II of the project.

3.1.4 Monitoring Site Selection

Monitoring sites will be selected based the results of previous research (Dailer, 2010; Hunt and Rosa, 2009) and the reconnaissance done by this study as described above, particularly via the TIR and Rn surveys. Additional site selection criteria will include the following:

- Warm sea surface temperature anomalies as measured the TIR survey
- Seepage water temperature
- Presence of black precipitate around the circumference of the submarine spring
- Discharge rate as measured with current meter
- Low salinity
- Low pH
- Low oxidation/reduction potential

The water quality parameters listed above will be measured with a YSI 600XL Water Quality Analyzer or the equivalent. Up to four sites selected for the most intense monitoring for the tracer dye emergence. Up to four additional sites will be selected and monitored but at a less frequent intervals than the primary sites. These are sites of possible dye emergence, but in the judgment of the principal investigators the probability that this occur is not a likely as the primary sites.

The final selection of the monitoring sites will be determined by the principle investigators of this project based on their experience. It is expected that a majority of the springs identified in by Dailer, and Hunt and Rosa will be designated primary sampling sites.

3.2 GROUNDWATER TRACER PROGRAM

3.2.1 Tracer Dye Selection

Many techniques exist for tracking the movement of groundwater using introduced or natural tracers. Stanley et al. (1980) specified that a tracer should:

- Be non-toxic
- Move with the flow of groundwater
- Be chemically stable over the duration of the tracer test
- If introduced not be naturally present
- Not be removed by natural filtration
- Be detectable at very low concentrations

There is no ideal tracer, but suitable candidates include ionic salts (Wood and Dykes, 2002; Levy and Chamber, 1987; and Olsen and Tenbus, 2004), fluorescent dyes (Smart and Laidlaw, 1977; Chua et al., 2007; Flury and Wai, 2004, and Sabatini, 2000), dissolved gases (Malcolm et al., 1980; and Wilson and McKay, 1993), radionuclides, and spores and bacteria (Davis et al., 1980, and Harvey, 1997). Ionic salts are attractive because they can be detected in low concentrations with ion specific probes. The most widely used are chloride and bromide salts. In this study interference from marine salts will be a problem. Chloride would be overwhelmed by seawater chloride. The bromide ion is present in Hawaii groundwaters at concentrations of 0.06 mg/L to 0.8 mg/L (Hunt, 2004) making addition of this ion an attractive secondary tracer. However, this program will be monitoring for the emergence of the tracer in submarine springs where a mixture of freshwater and recirculated seawater will be discharging. Seawater has a dissolved bromide concentration of 67 mg/L (Henderson, 1990). The high tracer concentration required to overcome the inference from seawater bromide made this option too expensive. The presences of dissolved gas tracers can be monitored for on-site and in low concentrations (Davis et al., 1980). However, the equipment is bulky and expensive. Radionuclides have safety and regulatory issues, while special techniques need to analyzer for spores and bacteria are not field friendly.

The tracer of choice for many studies is fluorescent dyes. They are non-toxic (Field et al., 1995), detectable to the parts per trillion concentrations with a fluorometer, many are stable, and tend remain in solution rather than sorbing to the aquifer matrix or suspended particulate matter. Due to possessing the fore mentioned desirable qualities the yellow-green dye fluorescein and the orange-red dye Rhodamine WT are most widely used of this class of tracers. Both can be analyzed by UH personnel at the Manoa campus and in the field using a handheld fluorometer. To test whether the suspected coastal effluent discharge problem is specific to an injection well group, two tracer substances are needed for this study, which will be fluorescein (FLT) and Rhodamine WT (RWT).

FLT is a yellow-green dye that has been used in tracer studies since the end of the 19th century (Smart and Laidlaw, 1977). FLT is non-toxic to humans and the environment at concentrations used in tracer tests (1 to 2 mg/L) (Field et al., 1995). This dye has the advantage of being relatively economical and widely available. A disadvantage for this study is that it has fluorescence characteristics that are similar to that of wastewater. The fluorescence couple for this dye is an emission wavelength of 490 nano-meters (nm) and emission wavelength of 520 nm, which is very close to that of wastewater. For example, Galapate et al. (1998) found fluorescence peaks at 524 nm for gray water and 531 nm for sewage effluent. This will necessitate a thorough background fluorescence investigation and a tracer concentration high enough to overcome the interference problems. FLT is unstable when exposed to artificial or natural light. This will prevent problems with dye coloring the nearshore waters, but will necessitate the collection of samples in dark colored or opaque bottles. Since the travel path for the tracer test is underground this will not be an issue from a detection perspective. However, it will be necessary to shield the samples from light once they are collected. Fluorescence of this dye also decreases at pH values less than 6.5 (Smart and Laidlaw, 1977). The pH of the waters sampled in this study is expected to be 7 or greater due to the seawater and carbonate reef material buffering.

RWT is a red dye that is commonly used in wastewater investigations. It has a fluorescence couple of 550 nm (excitation) and 588 nm (emission). This is significantly longer than that of wastewater effluent reducing interference problems and allowing a lower limit of detection. It is stable in waters with a pH higher than 5 (Smart and Laidlaw, 1977). It is less economical than FLT, but the lower detection limit can offset this disadvantage. Another consideration when using RWT is that it occurs in two isomers with differing sorption characteristics (Sutton et al., 2001). As the travel time of the tracer dye increases there will be a separation of the two isomers resulting in double peaked breakthrough curves. This will be difficult to interpret since multiple peaks could be caused by numerous other factors such as numerous paths that groundwater can take from the point of injection to the point of discharge. However, the first of any peaks detected will provide valuable information about the travel time from the LWRF to the submarine springs.

3.2.2 Background Fluorescence

Section 4.1.1 gives the procedures for collecting a sample from a submarine springs. This section describes the purpose and the interpretive methods used to establish a background fluorescence value(s) for the study site.

The fluorometers used in this study have manufacturer specified detection limits of 0.01 ppb for both FLT and RWT. However, natural and anthropogenic compounds in water mixture emerging from the submarine springs have fluorescence characteristics that may mimic that of the dyes selected for this study (Meus et al., 2006; and Smart and Karunaratne, 2002). These inferences include fabric brightener agents that fluoresce in the blue wavelengths (Poiger, 1998). Although these agents will be expected in the LWRF effluent, the blue wavelengths are well below that of the dyes used in this study. Other in-situ sources of fluorescence such as fluvic acids fall in wavelengths significantly shorter than that of FLT and RWT (Baker et al., 2003). More problematic are fluorescent peaks at about 520 nm that have been identified in at least two studies. Smart and Karunaratne (2002) attributed this peak to antifreeze containing FLT. In their study of the fluorescence of domestic wastes in the Kurose River in Japan, Galapate et al. (1998) showed there was a 531 nm peak in sewage effluent. When effluent was mixed with river water this peak shifted to a wavelength of 524 nm very close to that of FLT.

This potential interference raised significant concern during the development of this WP. To investigate this possible problem the fluorescence of R-2 water, raw groundwater, municipal tap water, and coastal seawater was measured using a Turner Designs 10AU Fluorometer with the FLT optics package installed. The R-2 water was collected from the Wahiawa Wastewater Treatment Plant (WWTP) located in Wahiawa, Oahu, Hawaii. The raw groundwater was collected from the Waipahu III Wells in Waipio, Oahu, Hawaii. This groundwater is located in an area where pineapple cultivation once dominated, similar to the groundwater in the Kaanapali area of West Maui, Hawaii. The seawater was collected from an exposed section of coastline located on southwest Oahu, Hawaii. As shown in Figure 3-2, the fluorescence (in raw fluorescence values) of tap water, groundwater, and seawater were very low and nearly identical. The R-2 water fluorescence was nearly identical to that of a 1 ppb FLT standard. The fluorescence of the 10 ppb FLT standard was over an order of magnitude greater than the R-2 water. Based on this preliminary assessment, FLT concentrations as low as 2 ppb should be detectable in R-2 water. The injection concentration of 5,000 ppb allows over three orders of magnitude dilution before the FLT signal is lost in the background fluorescence. This is a conservative estimate since there will be some the dilution with low background fluorescence seawater and fresh groundwater. A more comprehensive evaluation will be done to determine background fluorescence magnitude, the limit of detection, and background correction offsets.

3.2.2.1 Pre-Dye Addition Monitoring

Pre-monitoring is one of the recommended methods in characterizing background fluorescence (Smart and Karunaratne, 2002; and Meus et al., 2006). For this study, a two-week pre-dye monitoring period will be done to measure the magnitude and variability of in-situ fluorescence at the selected monitoring sites. As described in Section 3.2.5, up to four sites will be designated as primary monitoring sites and up to four additional sites will be designated as secondary monitoring sites. During this two-week period, samples will be collected daily from the four primary sites. Also, samples will be collected daily from two of the four secondary sites. The secondary sites sampled will be alternated so each secondary site is sampled every other day. The samples will be read for both FLT and RWT fluorescence. The mean and standard deviation of the fluorescence values for each sample location and for the entire sample population will be computed. The data will also be reviewed for temporal and spatial trends in fluorescence. The result of this analysis will be one factor used to establish the limit of detection (LOD). The methods that will be used for sample collection are described in Section 3.2.5 and Section 4.

3.2.3 Tracer Addition – Amount and Duration

The tracer dye needs to be introduced in a high enough concentration so that it is detectable at the sampling points. The primary processes that will reduce the concentration are dilution due to dispersion, retention of the tracer in the formation, dilution via mixing with seawater within the aquifer and at the sampling point (i.e. at the submarine spring), and tracer decay. Other factors that must be considered are potential stratification if the tracer solution density if greater than that of the effluent, and the cost of the tracer. Our preliminary MODFLOW/MT3D model indicates that dilution of up to three orders of magnitude could be expected if there is no preferential pathway between the effluent injection wells and the submarine springs. This is a worst-case scenario since the identified localized discharge at the submarine springs strongly suggests a preferential flow path does exist. Samples at the submarine springs collected by Hunt and Rosa (2009) had a seawater content that varied from 74 to 85 percent. We expect our piezometer sampling method to result in lower percentages of seawater. The manufacturer's stated detection limit for FLT and RWT using a Turner 10AU Fluorometer is 0.01 ppb in potable water. However, this study will be sampling treated effluent water with organic matter that may interfere with fluorometric analysis. Hence, we have chosen tracer concentrations will be will be detectable above background concentrations after undergoing three orders of magnitude of dilution. With the study area, preliminary limit of detection for FLT is about 2 - 3 ppb, while that for RWT is less than 1 ppb (based on the work of Tetra Tech, 1994).

This study will use an injection of two different tracer dyes. This approach is taken to assess the relative impact to the nearshore environment from each of the two well groups. The north well group will include Injection Wells 1 and 2. The south well group will include Injection Wells 3 and 4. Each tracer dye powder will be separately mixed with 50 gallons of fresh water in a 55 gallon drum. The drum will marked at 5 gallon increments up to 40 gallons, then at two-gallon increments up to 50 gallons. This will facilitate accurate mixing in the field. The powder will be added as the drum is filled to aid in dissolving the dye into the water. The minimum injection concentration (concentration in the effluent stream) for RWT will be approximately 3,000 ppb while that for FLT will be approximately 6,000 ppb. The weight of each dye required to attain this concentration is shown in Table 3-1 based on the manufacturer's specifications (Kingscote Chemicals technical specifications – Appendix A). Table 3-2 shows the volume of concentrate required for each dye and the mixing rate to attain a concentrate solution equivalent to that provided by the manufacturer. This table also shows the delivery rate of concentrate to the effluent stream needed to produce the desired tagging concentration.

Table 3-1. Weight of Dye Powder Required

Tracer Dye	Volume of water tagged to 1,000 ppb with 1 lb. of dye powder	Approximate Volume Effluent to be Tagged	Desired minimum concentration	Weight of dye required
	(gallons)	(gallons)	(ppb)	(lbs.)
RWT	60,400	2,000,000	3,000	100
FLT	120,000	2,000,000	6,000	100

Table 3-2. Volume of Dye Required and Dosing Rate

Tracer Dye	Lbs. of dye required	volume tagged to 1,000 ppb per gal. of concentrate	Volume of Concentrate Required For the Desired Concentration	Mixing Rate	Dosing Rate Required
	(lbs.)	(gal.)	(gal.)	(lbs./gal.)	(gal./hr./mgd)
RWT	100	25,000	240	0.42	10
FLT	100	100,000	120	0.83	5

The tracer will be added to the injectate at a rate to give a tracer concentration of 6,000 ppb for FLT and a concentration of 3,000 ppb for RWT. The average daily effluent injection rate at the LWRF for the previous three Julys has been 3.6 mgd. This plan assumes an injection rate of 2 mgd at the selected well group during the tracer dosing. Thus, an addition of 240 gallons of RWT and 120 gallons of FLT added over a period of approximately 24 hours will be required.

3.2.4 Addition Procedures

The concentrated dye solution will be mixed in plastic 55 gallon drums. The mixing will occur at least one-hour prior to addition to ensure that all dye powder is dissolved. A pump, metering valve, flowmeter, and solution agitator will be fitted to a plate that will be placed on top of the drum. The agitator will ensure the solution stays uniformly mixed during the period of injection. Prior to dye addition to the effluent stream, the rate of effluent injection will be checked at the LWRF control center. The dye pump will be started and the flow rate adjusted with the metering valve so that the dye concentration in the effluent is at the correct value for each dye.

This study will do a series of two injections on successive days. This approach will help determine the relative impact to the nearshore environment of Wells 1 and 2 (north well group) as opposed to Wells 3 and 4 (south well group). Figure 3-3 is a graph generated by the LWRF that shows a typical injection rate for the summer time. Wells 1 and 2 only received effluent during the peak water use times in the late afternoon and a short time in the morning. Wells 3 and 4 received effluent throughout the day with the total exceeding 2 mgd except for a few hours late at night. To accommodate the tracer addition modifications to the plant operation will be necessary.

The first addition will add the dye RWT to the south well group. The LWRF will inject effluent into Wells 3 and 4 until the disposal rate exceeds approximately 2 mgd. During this period the plant will need to modify its normal operations so that any flow exceeding 2 mgd will be diverted to the north well group. The dye will be injected directly into each well and the delivery rate to each well controlled by a metering valve and observed using a flow meter. The flow into each well in the south well group will be verified every 0.5 hours and adjustments made to the delivery rate accordingly. The desired concentration in the effluent for RWT will be 3,000 ppb. This will require 5 gallons per hour (gph) for every 1 mgd of effluent injection (a total of 10 gph, but split between the two wells based on the effluent injection rate). The time, well, dye delivery rate, and effluent injection rate will be recorded every 0.5 hours on Form A in Appendix B.

Dye addition to the north group Wells 1 and 2 will require a significant modification of plant operation. The desired effluent injection rate into the north well group is also 2 mgd. This will require restricting flow to the south well group so this injection rate can be maintained. FLT will be added to this well group at a target concentration of 6,000 ppb. This will require the delivery of 2.5 gph for every 1 mgd of effluent injection for a total delivery rate of 5 gph. The point of dye addition to the north well group will be effluent splitter box between Wells 1 and 2.

3.2.5 Submarine Spring Monitoring

Monitoring at the submarine springs will commence no later than 24 hours after injection is started. Section 4.1.1 describes the procedure to be followed to collect a sample. As described in Section 3.1.4, up to four primary sampling sites will be selected and up to four secondary sampling sites will be selected. The sampling frequency will be front loaded, in that immediately following tracer dye injection sampling will be done at closely spaced intervals. As the postdye-addition time increases the frequency of sampling will decrease. Table 3-3 gives the phase of the monitoring program and the frequency of sampling at the primary and the secondary sites.

Table 3-3. Submarine Spring Sampling Frequency

Monitoring Phase	Weeks Since Dye Addition	Primary Sites	Secondary Sites
Background	-2 through 0	Sample up to four sites once per day	Sample two sites once per day. Alternate sites sampled
Post Dye Addition	0 through 2	Sample up to four sites twice per day	Sample up to four sites once per day
	3 through 6	Sample up to four sites once per day	Sample up to two sites once per day. Alternate sites sampled
	7 through 16	Sample two sites every other day. Alternate sites so each site is sampled at least once every four days	Sample two sites every other day. Alternate sites so each site is sampled at least once every four days

3.3 DATA EVALUATION AND PRESENTATION

The tracer results will be interpreted by using both analytical quantitative and numerical approach. Objectives are to estimate aquifer parameters and tracer related characteristics.

3.3.1 Analytical Calculations

Hydraulic parameters for the aquifer are estimated by the method of moments. The zeroth moment is used to estimate the tracer mass recovery, the first moment is used to estimate the mean residence time and mean flow velocity, and the second moment is used to estimate the longitudinal dispersion. Analytical solutions are for the three-dimensional solute transport equation under simplification assumptions, including the assumptions of infinitely large, homogeneous aquifer under one dimensional flow field.

3.3.2 Breakthrough Curve

The report by Field (1999) and the software associated with it will be utilized in the analytical calculation. The results are applicable to karst or fractured rock media, which can be applicable to the site under consideration. Using the breakthrough curves, we will estimate the total tracer recovery, mean residence time, mean tracer velocity, longitudinal dispersivity, and tracer dilution. Empirical fluid dynamics models will be used to estimate the Peclet number and the Reynolds number. The description below is mostly based on the Field report.

3.3.2.1 Total Tracer Recovery

Estimation of tracer recovery for individual sampling stations is given by Equation (3-1):

$$M_O = \int_0^\infty C(t)\, Q(t)\, dt \tag{3-1}$$

in which M_o is tracer recovery, C concentration, Q is discharge, and t is time. The total tracer recovered is estimated from Equations (3-2):

$$M_T = \sum_{i=1}^{n} M_{O_i} \tag{3-2}$$

where M_T is total recovery and n is the total number of stations.

The equations assume complete mixing of the tracer substance with water, negligible dispersion effects, and that the tracer mass will ultimately exit the aquifer system completely at one or more downgradient receptors as a function of time and discharge.

The quality of the tracer experiment may be quantified in terms of mass recovered. Usually, the quality of the tracer experiment is given as percent of mass recovered.

3.3.2.2 Mean Residence Time

Mean tracer residence time is the length of time required for the centroid (gravity mass) of the tracer mass to traverse the entire length of the aquifer system, representing the turnover time for the aquifer. The centroid is generally not the same as the peak concentration of the tracer mass in the tracer-breakthrough curve, but the more the groundwater flow conforms to Fick's law, the less obvious the difference between the centroid and the peak concentration.

Mean tracer residence time is estimated from Equation (3-3):

$$\bar{t} = \frac{\int_0^\infty t\, C(t)\, Q(t)\, dt}{\int_0^\infty C(t)\, Q(t)\, dt} \tag{3-3}$$

With the standard deviation given by Equation (3-4):

$$\sigma_t = \left[\frac{\int_0^\infty (t - \bar{t})^2 \, C(t) \, Q(t) \, dt}{\int_0^\infty C(t) \, Q(t) \, dt} \right]^{1/2} \tag{3-4}$$

These equations assume that tracer residence time will vary from zero for instantaneous exit of the tracer mass from the aquifer system to infinity for tracer mass that is stored in micropores. They provide relevant information on the time required for the centroid of a nonreactive pollutant mass spilled near the injected tracer mass to reach a downgradient receptor. Mean tracer residence time may be estimated by summation algorithms.

For contamination studies, initial tracer breakthrough (i.e., first arrival) may be considered more valuable than the tracer residence time, although it may have little theoretical meaning. Initial tracer breakthrough provides ground water managers with an indication of the length of time a contaminant will take to be detected at a downgradient receptor. However, because of the effects of longitudinal dispersion, and inadequate sensitivity of current analytical methods at extremely low concentrations renders this situation meaningless.

3.3.2.3 Mean Tracer Velocity

Mean tracer velocity is a measure of the flow rate of the centroid of the tracer mass and is given by Equation (3-5) with a standard deviation given by Equation (3-6).

$$\bar{v} = \frac{\int_0^\infty \frac{x_s}{t} \, C(t) \, Q(t) \, dt}{\int_0^\infty C(t) \, Q(t) \, dt} \tag{3-5}$$

$$\sigma_v = \left[\frac{\int_0^\infty x_s \left(\frac{1}{t} - \frac{1}{\bar{t}} \right)^2 \, C(t) \, Q(t) \, dt}{\int_0^\infty C(t) \, Q(t) \, dt} \right]^{1/2} \tag{3-6}$$

in which x_s is the distance to the monitoring point. Equations (3-5) and (3-6) also assume that tracer residence time will vary from zero to instantaneous exit of the tracer mass from the aquifer system.

Apparent tracer velocity is a measure of the rate of tracer migration as a function of initial tracer breakthrough; it is obtained by dividing the distance traversed by the tracer cloud by the time of first arrival of the tracer dye. Mean tracer velocity provides substantially improved insight into aquifer functioning over apparent velocity.

3.3.2.4 Longitudinal Dispersion

Longitudinal dispersion is most commonly estimated using the second moment. Longitudinal dispersion, D_L, is obtained using Equation (3-7):

$$\frac{D_L}{\bar{v}\,x_s} = \frac{1}{2}\frac{\sigma_t^2}{\bar{t}^2} \qquad (3\text{-}7)$$

Equation (3-7) assumes that Fick's law is always applicable; that is, there is no anomalous behavior. In actuality, immobile zones (dead zones) are common, which cause a long tail.

Tracer Dilution

Longitudinal dispersion theory for a conservative tracer, released as a slug at t = 0 and x = 0 in aquifers where dispersion and advection are assumed to be one-dimensional, suggests that a uniform Gaussian distribution of the tracer concentration will occur in the direction of flow:

$$C(x_s, t) = \frac{M_{in}}{A\sqrt{4\pi D_L\,t}}\exp\left[\frac{(x_s - \bar{v}\,t)^2}{4D_L t}\right] \qquad (3\text{-}8)$$

3.3.2.5 Empirical Fluid Dynamics Models

<u>**Peclet Number**</u>

The Peclet number is a measure of the relative contribution of mechanical dispersion and diffusion to solute transport:

$$Pe = \frac{\bar{v}\,x_s}{D_{x_s}} \qquad (3\text{-}9)$$

<u>**Reynolds number**</u>

Significance of the fractures can be estimated from the Reynolds number:

$$N_R = \frac{\rho\,\bar{v}D_C}{\nu} \qquad (3\text{-}10)$$

DC is average fracture diameter. If the Reynolds number indicates flow to be in the laminar regime, then an equivalent hydraulic conductivity K for flow within the conduit (or conduit) may be calculated. For laminar flow in fractured system, equivalent K is obtained by

$$K = \frac{D_C^2 \rho g}{8\mu} \tag{3-11}$$

and for laminar flow, K is obtained by

$$K = \frac{n_e w^2 \rho g}{12\mu} \tag{3-12}$$

To determine the total recovery of tracer injected into an aquifer, the following steps are used.

Tabulate and plot the Concentration Subtract background tracer concentration. Plot the concentration of tracer recovered

Tabulate and plot the Discharge vs. time.

Integrate Recovery Curve Quantification of tracer recovery is found by numerically integrating underneath the tracer recovery curve. This is accomplished by setting up a table, similar to this one:

t	Q	C	C x Q	t x C x Q

Integrate Recovery Curve Again, integrating the recovery curve a second time, but this time including time t and dividing by the mass recovered (step 3 above) according to Equation (3-3), yields the mean residence time.

Calculate Tracer Mass Recovery When the table of values is complete, Equation (1) can be solved by summing column 4 and multiplying by a time conversion to yield units of mass only. Hence, the solution to Equation (3-1) is acquired in a simplified manner by

$$M_O = \int_0^\infty Q(t)C(t)dt \approx \sum_{i=1}^{n} Q_i C_i \Delta t_i$$

(3-13)

Calculate Mean Tracer Residence Time Mean tracer residence time is found by solving Equation (3). Equation (3) is solved by the same method that Equation (1) is solved; by simplified summation of the data. From the table sum the values in column five. Divide the mass obtained in step 5 above into this number to obtain units of time.

Calculate Mean Tracer Velocity Divide the distance traversed by the tracer cloud by the mean tracer residence time to obtain mean tracer velocity.

Repeat for Subsequent Sampling Stations Repeat the above steps for all springs in which the tracer was recovered.

Calculate Total Tracer Mass Recovery If several springs recovered the tracer, then sum the individual masses obtained for each spring together to obtain the total tracer mass recovered.

Calculate Percent Mass Recovered Calculate the percentage of mass recovered by dividing the quantity of tracer mass recovered by the quantity of tracer mass injected and multiplying by 100. Analytical Solutions of the Convection Dispersion Equation

USGS: http://pubs.usgs.gov/twri/twri3-b7/html/pdf.html

IGWMC. http://igwmc.mines.edu/software/alpha.html

3.3.3 Numerical Models

Three models for this site will be developed in this study, as described below. The first model neglects the density-dependent flow and only considers fresh-water movement. The saltwater interface will be used to specify the bottom boundary of the model. The second model simulates the density-dependent flow in a cross section through the site. This model is aimed at understanding the temporal evolution and spatial distribution of the salt water/fresh water transition zone. Information gained from these models will be helpful in building the third model, a detailed three-dimensional model of the site.

3.3.3.1 Modeling Objectives

The objectives are to estimate aquifer parameters and tracer related characteristics in the study area. In contrast to analytical approaches, numerical solutions are based on physically based equations that are more realistic. Hence, the tracer test results can shed lights on the aquifer and hydrologic conditions at the study site. We should realize however that there are great degrees of uncertainty regarding aquifer properties and chemical interactions in the aquifer. A major difficulty is related to the potential existence of preferential flow. The adopted modeling approaches are suitable for porous media and are also considered acceptable if the media can be treated as equivalent porous media. Models for discrete fractures are not readily available and their data requirements are not easy to satisfy.

3.3.3.2 Steady-State MODFLOW Model

The Modular Finite Difference Groundwater Flow Model MODFLOW (Harbaugh et al., 2000), developed by the U.S. Geological Survey, is a widely used program for simulating groundwater systems. In general, applicability of MODFLOW for this site is limited due to its inability to simulate density-dependent flow. However, the model will be used in this study as an initial step considering the relative ease of using the model in comparison to a variable density model. The centerline of the freshwater/seawater mixing zone is taken as a no-flow boundary representing the bottom of the model. Available data, with tracer test data will be used to further calibrate the model and reduce uncertainties in predictions. The parameters obtained by using the analytical approaches described in Section 3.3.1 above will also be used to constrain the parameters for the numerical model. The calibrated model can be used in the future for predicting contaminant levels for future episodes of accidental releases, or assessing the effects of potential changes in injection rates or concentrations on ocean contamination.

3.3.3.3 Unsteady-State MODFLOW and MT3DMS Models

The results of the steady state run will be used as the initial condition for this model which will be linked to the solute transport model MT3DMS (Zheng and Wang, 1999; Zheng, 2006) to simulate the tracer test experiment. Unsteady conditions will be utilized for both models. Available data from test results and those obtained from the analysis of the breakthrough curve will be used in MT3DMS calibration.

3.3.3.4 SEAWAT Cross-Section Model for the Site

The USGS model SEAWAT (Langevin et al., 2007) can be used for simulating groundwater accounting for potential contamination due to seawater/freshwater mixing. SEAWAT combines MODFLOW with MT3DMS in simulating three-dimensional variable density groundwater flow, along with multi-species solute and heat transport. Given the complex hydrogeologic setting of the site, with anisotropic aquifers and a freshwater/seawater interface, SEAWAT is considered an appropriate modeling code for accurately simulating groundwater flow.

As a step in building a site scale, density dependent model, a cross section through the site will be developed as a two-dimensional model with a 650 ft width. The objective is to analyze the density dependent flow, specifically the temporal evolution of the freshwater/seawater interface and its inland propagation in more detail. The two-dimensional model is also useful for estimating appropriate site specific parameters in the groundwater zone through calibration. These types of simulations can be of great help in building a three dimensional model for the island (Gingerich and Voss, 2005).

Following model calibration, the tracer test will be simulated with due consideration to appropriate scaling of well injections to match the use of a two-dimensional model. A rough estimate can be estimated based to running a path-line model to identify the fraction of the discharge confined to the width of the section.

3.3.3.5 SEAWAT 3-dimensional Model for the Site

The insights gained in the two modeling steps described above will be used to construct a fully three-dimensional model for the site. Again, the model will be calibrated based on the available data and results from the tracer test and accompanying breakthrough curve.

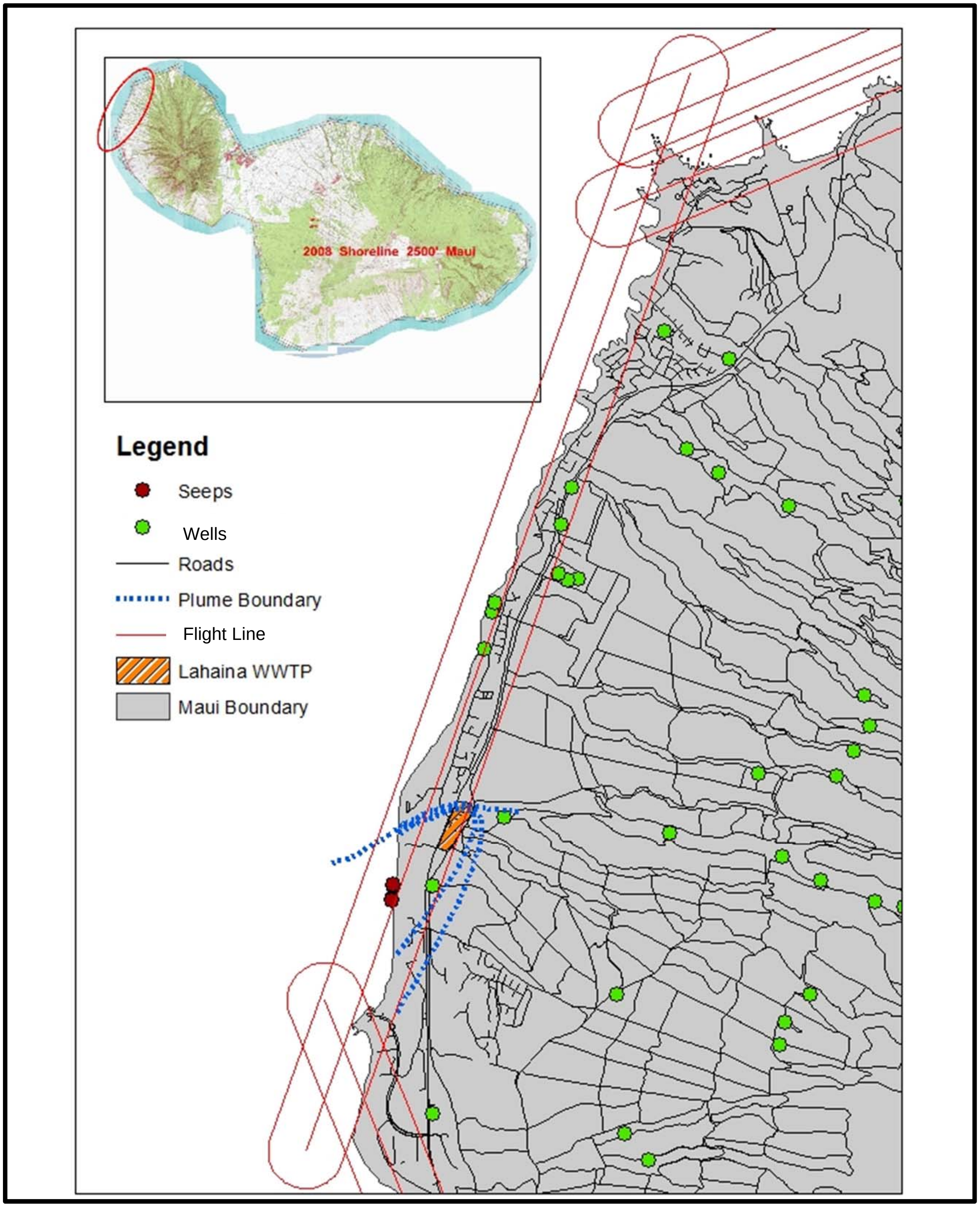

Figure 3-1. The Tracks of the TIR Flight Paths

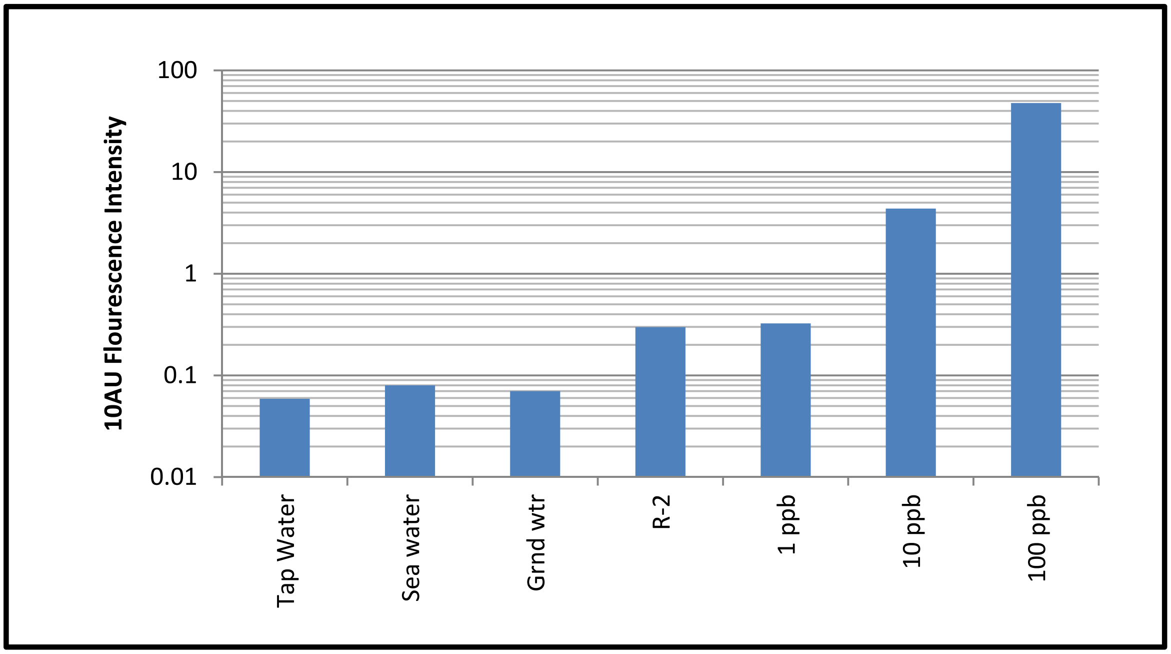

Figure 3-2. Measured Natural Fluorescence in Various Types of Waters Compared to FLT Fluorescence

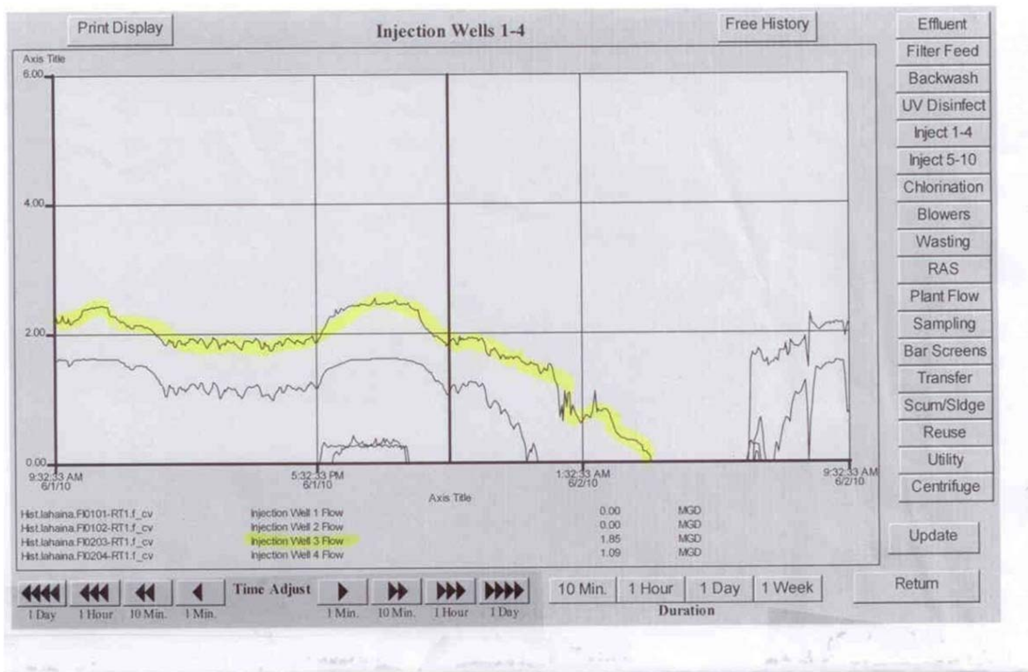

Figure 3-3. Typical Summer Time LWRF Injection Rates

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SECTION 4
SAMPLING AND QUALITY ASSURANCE PLAN

4.1 SAMPLING PROCEDURES

4.1.1 Submarine Spring Sampling Procedures

The sampling will be performed manually from a dinghy. The dinghy operator will navigate to the sample site and drop a weighted anchor that will not damage the reef below. Once the dinghy is suitably secured at the sample site, the sampling tube affixed to the previously emplaced semi-permanent piezometer will be retrieved from the ocean bottom and brought to the surface. It will then be fed through the rollers of an electric peristaltic pump. The pump will be started and the discharge initially directed into a purge bucket with volume markings. A volume equal to or greater than three times the volume of the stagnant water in the sampling tube and piezometer will be purged through the system prior to sample collection. The effluent from the pump will then be directed into a field analysis container and the pH, specific conductivity, temperature, and salinity of the sample water will be measured and recorded. Next the the sample bottle will be filled with the sample water. The sample container will consist of an opaque plastic to prevent photo-degradation of any tracer dye that might be present. The volume of sample collected will be no less than 60 ml. A 500 ml sample will be taken for every 19 samples collected in the smaller bottles to facilitate duplicate analysis and sample splits for other analyses. Once the sample bottle is filled to the shoulder it will be immediately capped to protect the sample from sunlight and spillage. A label will then be placed on the bottle containing the following information:

- Date of collection
- Time of collection
- Site name where the sample was collected
- Whether or not is was filtered
- Analysis to be performed (fluorescence)
- The name(s) of the sampler(s)

The information will be filled using an indelible pen such as a Sharpie^{R}. The sample bottle will then be placed in a suitable storage container (probably plastic cooler). The label will be sealed with transparent tape wrapping around the entire sample bottle. The sampling tube will then be disconnected from the pump, capped and returned to the ocean bottom.

4.1.2 Well Sampling Procedures

Groundwater and rain will be sampled from municipal production wells and rain gauges located across the Lahaina area in West Maui. Specific conductivity alkalinity, dissolved CO_2, dissolved O_2, and temperature will be measured in the field. EC and temperature will be determined on an YSI 63 Conductivity-Temperature meter and probe. Alkalinity and dissolved CO_2 will be measured using Hach digital drop titrators and reagents. Dissolved O_2 will be measured on a Hach DR/820 Colorimeter. pH will be measured with an Accumet AB 15 pH meter that will be calibrated against pH 4 and 7 buffers. Groundwater samples for laboratory analysis will be collected from pumps that ran for a least two-minutes prior to sampling to purge stagnant water from the well and the water delivery line. All water samples will be filtered through 0.45 μm cellulose nitrate filters. Filtered water will be collected in HDPE bottles filled to overflowing and stored at ~4 °C until analysis. The procedures for nutrient (e.g., nitrogen, phosphorous, etc.) sampling are included in Appendix D. Major ions, total organic carbon, total nitrogen and stable isotopes of carbon, nitrogen and oxygen will be analyzed at the University of Hawaii at Manoa. Major ions (Na, K, Ca, Mg, Cl, Br, F, NO_3, SO_4) will be measured using a Dionex DX-120 ion chromatograph. Dissolved SiO_2 will be determined via a Hach DR/4000 spectrophotometer. Dissolved organic carbon and dissolved total nitrogen will be measured on a Shimadzu TOC-V analyzer with TNM-1 detector. Nutrients will be analyzed at the University of Washington. $\delta^{13}C$ values of dissolved inorganic carbon will be measured following Torres et al. (2005) using a ThermoFinnigan DELTA plusXP mass spectrometer. Dissolved nitrate $\delta^{15}N$-$\delta^{18}O$ from nutrient sample splits will be analyzed at the UH Isotope Biogeochemistry Laboratory using denitrifying bacteria (*Pseudomonas Aureofaciens*) after the procedures of Casciotti et al. (2002). Nitrite will be removed from isotope sample splits per the methods of Granger et al. (2002) prior to analysis (cf. Casciotti et al., 2007; Cassiotti and McIlvin, 2007).

4.2 MEASUREMENT OF FLUORESCENCE

4.2.1 General Procedures

A pre-cleaned cuvette will be filled and emptied at least once with sample water to remove any residual rinse water. The final sample aliquot for analysis will be filtered using a syringe with a 0.45 micron filter attached. The syringe will be filled with a sample aliquot to amount slightly greater than the desired volume of the cuvette. The filter will be attached and a small amount of sample discharged out of the filter to clear the sample path. The cuvette will then be filled to the desired level and the fluorescence read with the fluorometer. It will then be filled again and the field fluorescence read using the procedures described in Appendix B, Section 3.6. At the beginning of each day that a fluorometer is to be used the accuracy will be verified with distilled water blank and a 10 ppb standard solution of each dye type. The results of the check will be recorded Two-Point Fluorometer Accuracy Form (Appendix B, Form B.

4.2.2 Measurement of Fluorescence in Field

Fluorescence will be measured with the handheld fluorometer in the field or as soon as practical thereafter. At the time of sample collection the sample temperature, pH, specific conductivity, and salinity will be measured and the results recorded on Form C in Appendix B. Field fluorescence readings will be performed using the procedures described in Appendix C, Section 3.6. The results will be recorded on Form D in Appendix B.

4.2.3 Measurement of Fluorescence in Laboratory

Fluorescence will also be measured in Laboratory. This is a more controlled environment and also provides the opportunity to conduct more quality assurance tests such as spiking the samples with known amounts of tracer dye and evaluation of the instrument recovery. The laboratory measurements will be done with a Turner Designs 10AU Fluorometer. Fluorescence read using the procedures described in Appendix C, Section 3.G. The results will be recorded on Form E in Appendix B.

4.3 EQUIPMENT DECONTAMINATION

All equipment coming in contact with samples and that are reused during sampling will be decontaminated to prevent carryover of any dye. The reused equipment will include syringes used when filtering aliquots, cuvettes used if measuring fluorescence, and primary dye sample bottles. When going to the field, a sufficient number of syringes and sample collection bottles will be taken so no decontamination in the field is needed. Once used, the equipment will be segregated from the unused sampling equipment in a cooler marked “USED” so accidental re-use does not occur in the field. Upon return to the lab or maintenance area used equipment will be cleaned as soon as possible with a mixture of potable water and detergent. The detergent will be Alconox^{R} or another approved laboratory detergent. The equipment will be rinsed with potable water. Finally, the equipment will be given a final spray rinse with distilled water then placed in a rack to dry. Dye sample syringes will be cleaned by submerging the tip in the water and pulling back on the plunger. Then pushing plunger back into the syringe barrel as far as it will go to expel wash or rinse water. Geochemical sampling procedures are outlined in Appendix E.

The cuvettes used for fluorescence measurements in the field will be decontaminated in the field by rinsing the cuvettes in seawater at least three times. Then using a household sprayer filled with distilled water to thoroughly rinse them.

4.4 SAMPLE HANDLING

4.4.1 Sample Volumes, Container Types, and Preservation Requirements

This is covered in Appendix E.

4.4.2 Sample Identification

A label will then be placed on the bottle containing the following information:

- Date of collection,
- Time of collection,
- Site name where the sample was collected,
- Whether or not is was filtered,
- Analysis to be performed (fluorescence), and
- The name(s) of the sampler(s).

This information will also be recorded on the daily field sampling form, Form C in Appendix B.

4.4.3 Sample Shipping

If samples are shipped to a laboratory for analysis they will be packed securely in sturdy container. For samples that must remain cold, a cooler will be used and a sufficient quantity of chemical ice packets inserted around the samples to ensure the temperature is equal to or less than 4 ^{o}C at the time it is received at the laboratory. All shipments of samples will be accompanied by a Chain of Custody Form (Appendix B, Form F). It shall be signed prior to sealing the cooler, and the airbill and shipping method noted. Samples that are to be analyzed for fluorescence do not need to be chilled, but must be protected from light.

4.5 FIELD QUALITY CONTROL

Organic matter may fluoresce in a manner similar to the tracer dyes causing interference during sample analysis (Mues et al., 2006; and Smart and Karunaratne, 2002). This interference needs to be corrected for. This process consists of directly measuring the fluorescence of treated effluent tagged with tracer at various concentrations and measuring the fluorescence of the submarine springs samples collected for a period of two week prior to dye addition. The spiking of treated effluent with tracer dye followed by analyzing the spiked effluent samples at different dilutions will establish the response of the fluorometer to effluent versus tracer dye concentrations. Analyzing pre-dye injection samples collected at the submarine springs will establish the background fluorescence of the study site.

4.6 LABORATORY BACKGROUND FLUORESCENCE EVALUATION

To evaluate relative fluorometer response to the treated effluent versus the tracer dye the fluorescence a matrix of effluent and tracer dye strengths will be evaluated. Effluent treated to R-2 standards (the effluent grade injected at the LWRF) will be collected from the Wahiawa Wastewater Treatment Plant on Oahu. Dilutions with raw groundwater and coastal seawater will be performed to replicate the range of water compositions expected to be collected at the submarine springs. Then, a series of mixtures of effluent, freshwater, and coastal seawater will be created in the relative proportions of the three source waters as detailed in Table 4-1.

Table 4-1. The Composition of the Background Fluorescence Study Laboratory Standards

Effluent	FW/SW Sample 1	FW/SW Sample 2	FW/SW Sample 3	FW/SW Sample 4	FW/SW Sample 5
(%)	(%/%)	(%/%)	(%/%)	(%/%)	(%/%)
100	NA	NA	NA	NA	NA
50	50/0	25/25	0/50	NA	NA
10	90/0	60/30	45/45	30/60	0/90
FW – raw groundwater SW – coastal seawater					

These aliquots will be divided into four subsets and individual samples will be spiked with the appropriate amount of tracer dye to create standards containing 0, 1, 10, and 100 ppb of the tracer. The fluorometer will be calibrated using the procedures described in Section 4.6.1 with tracer dye standards of 0.1, 1, 10, and 100 ppb concentrations of tracer dye in distilled water. The effluent fluorescence evaluation standards will then be measured using the calibrated fluorometer. The results will be graphed as a family of curves of tracer dye concentration versus fluorometer response. The evaluation of the measurements will be done based on the closeness of the effluent sample response to standard calibration curve. Where the linearity of the two responses is parallel (on a semi-log scale) an offset will be computed. It is expected that as the effluent concentration increases and the tracer dye concentration decreases the slope of the lines will no longer be parallel to the standard calibration line. Where the response lines are no longer parallel, indicates the point at which background fluorescence starts to provide significant interference with the detection of the dye. At this point, a single offset to correct for background is no longer adequate to accurately quantify the dye concentration. The results of this evaluation will be factored into the final determination of limit of detection for this study.

4.6.1 Quality Assurance of Fluorescence Measurements

4.6.1.1 Preparation of Calibration Standards

Calibrations standards of 0.0, 0.1, 10, 100, and 1,000 ppb will be prepared for calibration and as stock solutions for the limit of detection (LOD) and background assessments. This will be done using serial dilutions of an initial 100,000 ppb solution.

The initial solution for RWT will be made by adding 0.5 ml of 20 percent active ingredient dye solution to 1 L of distilled water. The dye solution will be measured using a 1 ml pipette with a precision of 0.05

ml. The contents of the pipette will transferred to a 1 L volumetric flask that is filled with distilled water to just below the 1 L mark. Once the dye aliquot has been transferred to the volumetric flask, distilled water will be added to bring the level up to the 1 L mark. A similar stock solution will be made for FLT based on its active ingredient content. This stock solution will then be used to make the calibration standards calibration of the fluorometer. This will be accomplished by completing a series of dilutions of the 100,000 ppb stock solution. The serial dilutions will be limited to two so as to minimize the propagation error. This stock solution will then be diluted as follows:

- A 1,000 calibration solution will be made by diluting the stock solution by a factor of 100 using a 1L volumetric flask and a 10 ml graduated cylinder;
- A 100 ppb calibration standard by diluting the stock solution by a factor of 1,000 using a 1 L volumetric flask and a 1 ml pipette. This calibration will also serve as a stock solution to make the 300, 100, 10, 1, and 0.1ppb calibration standards.
- The remaining calibrations standards except distilled water blank will be done in the same manner as the preceding standards except that the 100 calibration standard will be used as the stock solution.

Distilled water only will be used for the 0 ppb calibration standard. The standards will be decanted amber glass or plastic bottles that have been washed with a 20 percent bleach solution followed by a triple rinsed with distilled water. The bottles will be stored at room temperature and out of direct light.

4.6.2 Tracer Dye Analysis Quality Assurance Program

4.6.2.1 Establishing the Limit of Detection and Background Correction

The limit of detection (LOD) or method detection limit is defined as the concentration of a substance that can be measured and reported with a 99 percent confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the analyte (40 CFR Appendix B to Part 136, [Appendix D of the WP]). For this study the analytes will be FLT and RWT and the matrix will be the submarine spring discharge water. This assessment will be done in four steps:

1. Measure the reproducibility of the fluorometer by analyzing at least seven distilled water blanks. Each analysis will be done in the same manner as a series of sample analyses. Each sample will be read in different cuvettes, and filtered and decanted into the cuvettes in the same manner as would be done for a normal sample. The mean and standard deviation of the distilled water blank measurements will be computed to estimate the fluorometer noise.

2. The fluorescent for the population of the background samples collected at the submarine springs will be measured for both FLT and RWT fluorescence. As in Step1, the mean and standard deviation of the fluorescence measurements will be computed. Concentration units will be equivalent dye fluorescence (ppb FLT or ppb RWT).

3. Extractions from approximately twenty percent of the background samples will be composited. The volume of the composite will be great enough to generate five spiked samples. Two of the composite samples will be spiked to concentrations near the expected LOD. The first sample will be spiked to a dye concentration equivalent the mean background fluorescence plus 2 standard deviations. The second sample will be spiked to a dye concentration equivalent the mean background fluorescence plus 5 standard deviations. These two spiked samples will be used to compute the LOD. The other samples will be spiked to concentrations of 10, 100, and 300 ppb. These additional spiked samples plus the LOD samples will be used to detect inflections in the slope of the fluorometer response that will indicate when background fluorescence and instrument noise start affecting real dye signature.

4. LOD analysis will be done for two lowest concentration spiked samples using the procedures documented in 40 CFR Appendix B to Part 136 (Appendix D of this WP) for computing a method detection limit.

5. The final LOD will be compared to inflection points the slope of the graphed instrument response line. The inflection point should plot at a higher concentration and will indicate the practical quantification limit (PQL). Any sample concentration that falls between LOD and the PQL will be reported as trace amount detection. A concentration falling below the LOD will be reported as non-detectable.

This series of analyses will provide a background correction estimate. The fluorometer is zeroed using a distilled water blank. It is then calibrated using a calibration standard generated using dye and distilled water (Section 4.6.2.2). The fluorescence of a field sample will roughly be the sum of the natural fluorescence plus the dye fluorescence (Meus et al., 2006). Thus a basic correction for background fluorescence can be made by subtracting mean background fluorescence value from the sample fluorescence. However, since the background fluorescence may vary in time and space this is only an approximation. Reporting of dye fluorescence will include the total, background mean, and corrected fluorescence.

4.6.2.2 Fluorometer Calibration

Fluorometer Calibration – Aquafluor Handheld

The instrument response will be tested with calibration standards that include the concentration expected at the submarine springs and the planned injection concentration. This instrument has a linear range of 0 to 300 ppb. Beyond this range the instrument response becomes non-linear and can decrease with increasing concentration due light absorption. This will occur at approximately 3,000 ppb. Samples collected at the submarine springs will not be expected to exceed the linear range. However, dye tagged effluent samples will exceed the linear range and will require dilution prior to being read with the fluorometer. Samples that have visible concentrations of dye will be read with the fluorometer, then diluted by a factor of 10 and read again. A proportional decrease (i.e. a fluorescence reading that is one-tenth of the original reading) shows that the previous sample was within the linear range. If the original sample was not in the linear range, the sample will undergo a second 1:10 dilution to bring the sample into the linear range. The recorded fluorescence concentration will be the final reading times the dilution factor needed to bring the aliquot into the linear range.

Calibration of this fluorometer will occur with the calibration standards as described in Section 4.6.1. This will test the fluorometer's response at seven different points using standards with concentrations of 0, 0.1, 1, 10, 100, 300, and 1,000 ppb. The 0 ppb calibration standard will be a distilled water blank. A two-point calibration will be done in accordance with the procedures documented in Section 3.4 of Appendix C. The first calibration point will be the 0 ppm distilled water blanks. The second calibration standard will be the 300 ppb standard. This will calibrate the instrument to the upper and lower bounds of its linear range. Once the two-point calibration has been completed, a complete set of standards from 0 to 1,000 ppb will be read. This will provide a series of reference data points that include the linear and non-linear range of the instrument. If cuvettes are be reused during the calibration process, the previous calibration aliquot will be cleaned using the procedures in Section 4.2 prior to the introduction of a new calibration solution aliquot.

New calibration standards will be needed throughout the project and will be prepared prior to completely expending the older standards. The consistency between the previous and new calibration standard will be checked by reading and aliquot of the old and the new standards sequentially. Any difference greater than 5 percent will be investigated and may require the generation of a new set of standards.

The seven-point accuracy check of the instrument will be done once a week. A two-point calibration that includes the 0 ppb blank and the 300 ppb standard will be done each day before sampling. Each calibration and accuracy check will be recorded on Form G in Appendix B.

Fluorometer Calibration – Turner 10AU

<u>Goals of calibration</u>

The goal of this calibration is to establish confidence that the fluorescence readings from the fluorometer over a wide range of concentrations. Accuracy is needed at very low concentrations to verify the presence of absence of dye in the samples and to quantitatively analyze the tracer breakthrough curve. Accuracy is required at high concentrations to verify the injection concentration. Thus, the instruments need to be challenged over a range of 0 to 1,000 ppb.

Section 3 of Appendix F details the calibration of the Turner 10AU fluorometer. The procedure that follows will reference this section for instrument specific operations while detailing the procedures specific to this project. Calibration involves six steps:

1. Turning the instrument on to allow the electronics and optics to come to a stable temperature.
2. Setting the instrument sensitivity.
3. Zeroing the instrument with a dye free blank.

4. Setting the linear range slope by adjusting the instrument response to match calibration standard of a known concentration.
5. Challenging the instrument with several calibration standards to verify the linearity of the response up to 100 ppb and to characterize the instrument response above the linear range up to the highest concentration expected to be tested.
6. Develop an instrument response curve for the instrument that covers the entire range of dye concentrations to be analyzed.

Cautions and considerations

For proper calibration the instrument must be allowed a 10 minute warm up time to allow the electronics and the optics to come to a stable temperature. Fluorescence is a temperature sensitive phenomenon, so the calibration standards and the procedure need to be performed at the same temperature at which the dye analyses will be performed. The cuvettes used during calibration will have the same optical properties as those used during the dye analyses. A series of blank analyses (a cuvette filled with distilled water) will be run to determine the consistency of readings with all the cuvettes used. This sub-procedure may be further optimized by placing a temporary mark near the top of the curvette. The cuvette will be placed in the cuvette hold with the mark facing away from the instrument panel. A fluorescence reading will be taken, and then the cuvette rotated 90 degrees and another reading taken. This will be repeated two more times, recording the fluorescence value at each orientation. An optimum orientation for the sample cuvettes can be determined by reviewing the results and finding the position for each that provides the most consistency throughout the population of cuvettes. That position will be marked with at the top lip with a permanent marker. For subsequent fluorescence analysis, the cuvette will be placed in the holder with the permanent mark facing away from the instrument panel. Also, for consistency of measurements, the outside of the cuvette needs to be cleaned with soft lint free cloth. For stubborn stains the cloth should be moistened with a solution of water and laboratory grade detergent. Then rinsed off with distilled water and dried with the soft lint free cloth. To avoid interference from room light or sunlight, the light cap needs to be securely in place over the cuvette holder.

Fluorescence is temperature sensitive. So it is important that all standards and blanks have equilibrated to room temperature prior to any measurements or adjustments. This temperature needs to be recorded on the Calibration Form (Appendix B, Form G).

Set the Instrument Sensitivity

The sensitivity of the instrument needs to be set prior to running a calibration for the first time. This will establish the maximum concentration that the instrument will read. This is accomplished by setting all calibration controls to their default values and reading the fluorescence of a prepared standard with a concentration equal to half of the maximum fluorescence concentration expected to be analyzed. For this study the critical samples will be those collected at the submarine springs and are expected to have a very low dye concentration. However, samples of the tracer tagged effluent will also be analyzed to verify injection concentration. These samples will likely have a dye concentration up to four orders of magnitude greater than that at the submarine springs. Since the critical operating range is biased to the low concentrations, the sensitivity will be set using a standard with a concentration of 10 ppb. The maximum measureable concentration will be 100 ppb, the limit of the instrument linear range. This will require that injectate samples be diluted by factor of 50 prior being analyzed in the fluorometer. Appendix 6B of the *Turner 10AU Fluorometer User's Manual* (Appendix E) details the steps to set the sensitivity.

Once set, the sensitivity does not have to be adjusted unless a different size cuvette is used or the lamp or optics are changed. Any changes to sensitivity will require a complete recalibration of the instrument.

Blank

The next step is to set the zero for the instrument. To accomplish this, dye free sample is read by the fluorometer to establish a zero point. The blank will be prepared using distilled water. Correction of sample background fluorescence will be done manually after samples are read based on the results of the background fluorescence study (Section 3.2.2). The blank needs to be set prior to calibration using calibration standard (10 ppb). If the instrument is later calibrated using a standard of a different concentration the blank does not need to be re-run. However, if the blank is reset another calibration is required. Steps required to set the blank are detailed in the *Turner 10AU Fluorometer User's Manual* (Appendix E) Section 3.F.3 steps 1 through 9D. While analyzing the blank, the instrument will not be set to subtract the blank value from the measured fluorescence concentration (Step 8).

Standard Calibration

The standard solution will adjust the slope of the analyzer response. The Standard Solution will be prepared in the detailed in Section 4.6.1.1 using distilled water. Since the expected dye concentrations at the discharge of the submarine springs will be low, the Standard Solution concentration will be 10 ppb. Steps required to set the standard calibration are detailed in the *Turner 10AU Fluorometer User's Manual* (Appendix E) Section 3.F.3 steps 10A through 10D.

Calibration Curve Development

As with the Aquafluor Handheld fluorometer, standards with a concentration of 0, 0.1, 1, 10, 300, 500 and 1,000 ppb will be used. The 0 ppb calibration standard will be a distilled water blank. Testing each of these standards sequentially will verify the linearity of the instrument response in the range of dye concentrations expected at the submarine springs and will provide a reference point for sample analysis beyond the linear range. The first calibration point will be the 0 ppm distilled water blank. If cuvettes are be reused during the calibration process the previous calibration aliquot will be cleaned using the procedures in Section 4.2 prior to the introduction of a new calibration solution aliquot. The samples will be read following the procedures specified in *Turner 10AU Fluorometer User's Manual* (Appendix E) Section 3.G. The concentration of the standard and fluorometer reading will be recorded on Form G in

Appendix B. The error at each point will be computed. If the error is systematic, a correction factor will be calculated and applied to all sample readings. The correction factor can be calculated by graphing the indicated fluorescence on the x-axis and the concentration of the associated calibration standard on the y-axis. A regression analysis will provide the best fit instrument correction equation. Figure 4-1 shows an example of a regression derived correction equation. This is best done in two steps. First, concentrations 100 ppb and below to checked to evaluate the linear range of the fluorometer and develop a further correction functions that are needed. Then concentrations 100 ppb and above will be checked to evaluate the non-linear range and a correction function developed to convert the fluorometer reading to true tracer dye concentrations. All the tracer dye concentration in the submarine spring are expected to all be less than 100 ppb so the linear range evaluation/calibration is the most important for this study.

4.6.3 Quality Assurance of Field Measurements

The primary field instrument other than the handheld fluorometer will be the YSI 63 pH and conductivity meter. The operations manual in Appendix G details the calibration and maintenance procedures for this instrument.

4.6.3.1 Calibration of the YSI 63 Meter

The procedures to calibrate the YSI 63 pH and conductivity meter are detailed Appendix G Section 4.2 for pH. At a minimum, we will use a two-point calibration with pH 7 and pH 10 standard solutions. These standards will be within their expiration dates. It is recommended that a portion of the standard be decanted to a clean 250 ml sample bottle. Repeated use of the pH standard can result in degradation. So it is further recommended that the pH standard in the 250 ml sample bottles be renewed at least monthly. A plastic 100 ml graduated cylinder will be used for the pH standard during calibration of the YSI 63. The accuracy of the meter shall be checked daily during field operations. The probe will be rinsed thoroughly with freshwater and dried to the extent practical when shifting from one pH solution to the other.

Appendix F, Section 4.3 details the procedures for calibrating the conductivity probe. The meter can only be calibrated at one conductivity value, but the accuracy of a wide range of conductivity values needs to be verified. The meter shall be calibrated using a standard solution with a conductivity of approximately 1,000 µs/cm. The accuracy of the meter at high conductivity values will be verified using a standard solution of approximately 50,000 µs/cm (a value close to the conductivity of seawater). If the difference at the high conductivity value is greater than 5 percent a correction factor will be computed. This will be done using the equation 4-3:

$$CF = (std\_hi-std\_low)/(mtr\_val-std\_low) \qquad (4\text{-}1)$$

Where:

CF = the correction factor (unitless)

Std_hi = value of the high range conductivity standard (µs/cm)

Std_lo = value of the low range conductivity standard (µs/cm)

The meter reading will then be corrected using the following equation:

mtr_corr = (mtr_val-std_lo) * CF + std_lo (4-2)

Where:

mtr_corr = the corrected conductivity reading (µs/cm)

It is important that the probe be thoroughly rinsed and dried when moving it from one solution to another to prevent carry over that will degrade the calibration accuracy of the standard solution.

All calibrations and daily accuracy assessments will be recorded on the Form H in Appendix B.

4.6.3.2 Maintenance of the YSI 63 Meter

Section 6 of Appendix G details the maintenance procedures for this instrument. Since it will be used in saltwater it is very important that the housing and probe be thoroughly cleaned at the end of each field day. Also the integrity of the o-ring for the battery compartment needs to be inspected and replaced is any damage is evident when changing batteries.

4.6.4 Quality Control Samples

4.6.4.1 Field Duplicate Samples

Field duplicates are two (or more) field samples taken at the same time in the same location. They are intended to represent the same sample population and are taken through all steps of the analytical procedure in an identical manner. These samples are used to assess precision of the entire data collection activity, including sampling, analysis, and site heterogeneity.

Duplicate samples are collected simultaneously or in immediate succession, using identical recovery techniques, and are treated in an identical manner during storage, transportation, and analysis. The samples may be either collocated samples or subsamples (“sample splits”) of a single sample collection. The sample containers are assigned a unique identification number in the field. Specific locations should be designated for collection of field duplicate samples prior to the beginning of sample collection. A minimum of one duplicate or replicate sample shall be included each week during the sampling program. Any duplicate sample analysis with a difference greater than 10 percent will have that difference noted in the remarks and a “D-“ flag qualifier listed in the results table.

4.6.4.2 Lab Control Sample

The Lab Control sample (LCS) is a sample of known composition prepared using dye-free water. Each LCS shall be spiked at a level less than or equal to the midpoint of the calibration curve (approximately 50 ppb). The LCS shall be carried through the complete sample preparation and analysis procedure. At least one LCS shall be prepared and analyzed for each dye (FLT and RWT) once each month during the sampling program.

4.6.4.3 Field Sample Spike

A field sample spike (FSS) is an aliquot of sample spiked with known concentrations of all target analytes. The spiking occurs prior to sample preparation and analysis. Each FSS shall be spiked at a level less than or equal to the midpoint of the calibration curve for each dye. The LSS is used to document potential matrix effects associated with a site.

4.6.5 Quality Control Limits

Any spikes sample with a less than 80 percent recovery rate will be reported with a <RC flag. All fluorescence analysis that occurred since the previous LCS or FSS with a recovery rate greater than 80 percent and prior to a successful QA check will also be flagged with <RC flag.

4.7 REPORTING REQUIREMENTS

4.7.1 Milestone Updates

Milestones for the project include:

1. *Field Reconnaissance* – Commence with the TIR flight during the week of 30 May. UH will request verbal concurrence to do this survey if the work plan has not been approved by this date.

2. *Develop a Tracer Study Design and Work Plan* – We are submitting this draft work plan by 10 June. Upon review of the work plan by the U.S. Army Corp of Engineers, the Hawaii Department of Health, and the U.S. Environmental Protection Agency; we will address the comments and submit a final work plan within 7 working days of receipt. *Review of Literature, Research Publications, and Prior Studies* will be done concurrently with the Work Plan development.

3. *Background Assessments* will begin on 20 June and continue through the addition. During this time, background fluoresce sampling, the radon/radium survey, and the geochemical/nutrient sampling will be done.

4. *Dye injection* – will commence on 5 July or upon the approval of the work plan plus two weeks. Submarine spring sampling will continue until at least 30 September. UH will inform HDOH, the USACE, and the USEPA at least one week prior to dye addition.

5. *Nutrient/geochemical and radon/radium survey*, Round 2 – will commence at the time of dye detection in the marine environment, with field studies completed approximately one-week later.

6. *Preliminary analysis and interpretations* – will be delivered by 7 October.

7. *Draft Report* – will be delivered by 11 November.

8. *Final Report* – will be delivered by December 23, 2011.

Notification of schedule changes will be made at least 72 hours prior to the planned time for the event being rescheduled.

UH will also provide quarterly progress to all program managers either by email or through formal meeting.

4.7.2 Draft Tracer Study Report

At the conclusion of the study UH will produce a compressive draft report that will provide an accurate, unbiased, and scientifically-defensible information related to the hydrologic connection between effluent discharges from LWRF and coastal waters. This report will be reviewed by HDOH, the USACE, and USEPA. The review comments will addressed to the mutual satisfaction of all of the organizations involved.

4.7.3 Final Tracer Study Report

The review comments from the review of the draft report will be incorporated into a final tracer study report

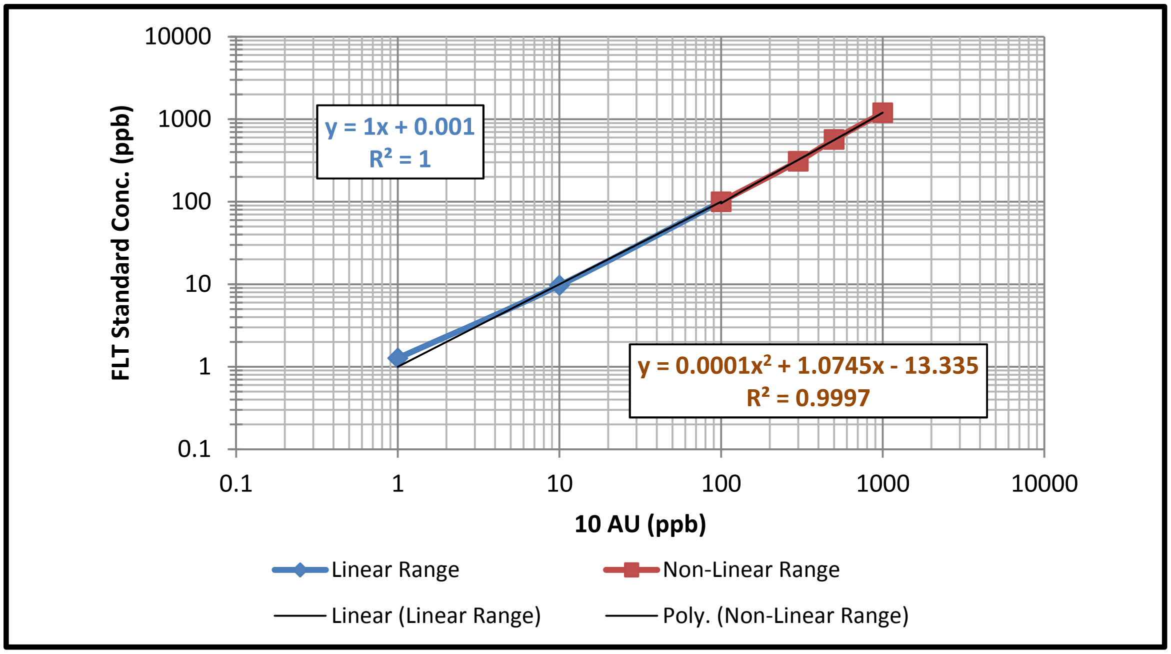

Figure 3-4. Typical Calibration Graph With Regression Functions

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APPENDIX A

Tracer Dye Technical Specifications

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Division of Kingscote Chemicals

WATER TRACING DYE
FLT YELLOW/GREEN PRODUCTS

TECHNICAL DATA BULLETIN

Bright Dyes Yellow/Green products are specially formulated versions of Xanthene dye, certified by NSF International to ANSI/NSF Standard 60 for use in drinking water. This dye is the traditional fluorescent water tracing and leak detection material and has been used for labeling studies from the beginning of the century. It may be detected visually, by UV light and by appropriate fluoremetric equipment. Today it is most often used visually. This dye has been used by the military to mark downed pilots for search and rescue operations over large water bodies. Visually the dye appears yellow/green, depending on its concentration and under UV light as lime green.

Based on biochemical oxygen demand (BOD) studies, the dye is biodegradable with 65% of the available oxygen consumed in 7 days. The dye is resistant to absorption on most suspended matter in fresh and salt water. However, compared to Bright Dyes FWT Red products it is significantly less resistant to degradation by sunlight and when used in fluoremetry, stands out much less clearly against background fluorescence. As always the suitability of these products for any specific application should be evaluated by a qualified hydrologist or other industry professional.

General Properties	Tablets	Liquids	Powders
Detectability of active ingredient [1]	Visual <100 ppb	Visual <100 ppb	Visual <100 ppb
Maximum absorbance wavelength [2]	490/520 nm	490/520 nm	490/520 nm
Appearance	Orange convex 1.6cm diameter	Reddish, brown aqueous solution	Orange fine powder
NSF (Max use level in potable water)	6.0 ppb	10.0 ppb	1.0 ppb
Weight	1.35 gms ± 0.05		
Dissolution Time [3]	50% < 3 minutes 95% < 6 minutes		50% < 3 minutes 95% < 6 minutes
Specific Gravity		1.05 ± 0.05 @ 25° C	
Viscosity [4]		1.8 cps	
pH		8.5 ± 0.5 @ 25° C	

Coverage of Products	One Tablet	One Pint Liquid	One Pound Powder
Light Visual	605 gallons	125,000 gallons	1,200,000 gallons
Strong Visual	60 gallons	12,500 gallons	120,000 gallons

Caution: These products may cause irritation and/or staining if allowed to come in contact wit the skin. The use of gloves and goggles is recommended when handling this product, as with any other dye or chemical.

[1] In deionized water in 100 ml flask. Actual detectability and coverage in the field will vary with specific water conditions.
[2] No significant change in fluorescence between 6 and 11 pH.
[3] (One tablet, 1 gram of powder), in flowing deionized water in a 10 gallon tank.
[4] Measured on a Brookfield viscometer, Model LV, UL adapter, 60 rpm @ 25° C.

Kingscote Chemicals, 3334 S. Tech Blvd., Miamisburg, Ohio 45342
Telephone: (937) 886-9100 Fax: (937) 886-9300 Web: www.brightdyes.com

MSDS PREPARATION INFORMATION

PREPARED BY:	T. P. MULDOON (937) 886-9100
DATE PREPARED:	1/1/08

PRODUCT INFORMATION

MAUNFACTURED BY:	KINGSCOTE CHEMICALS 3334 S. TECH BLVD. MIAMISBURG, OHIO 45342
CHEMICAL NAME	FLUORESCEIN, DISODIUM
CHEMICAL FORMULA	C(20)H(10)O(5) – 2Na
CHEMICAL FAMILY	XANTHENE DYE FORM

HAZARDOUS INGREDIENTS

NONE PER 29 CFR 1910.1200

PHYSICAL DATA

PHYSICAL STATE	POWDER
ODOR AND APPEARANCE	ORANGE/RED COLOR WITH NO APPARENT ODOR
SPECIFIC GRAVITY	~ 0.6 g/cc
VAPOR DENSITY (mm Hg @ 25 ° C)	NOT APPLICABLE
VAPOR DENSITY (AIR =1)	NOT APPLICABLE
EVAPORATION RATE (Butyl Acetate = 1)	NOT APPLICABLE
BOILING POINT	NOT APPLICABLE
FREEZING POINT	NOT APPLICABLE
pH	NOT APPLICABLE
SOLUBILITY IN WATER	HIGHLY SOLUBLE

FIRE HAZARD

CONDITION OF FLAMMABILITY	NON-FLAMABLE
MEANS OF EXTINCTION	WATER FOG, CARBON DIOXIDE, OR DRY CHEMICAL
FLASH POINT AND METHOD	NOT APPLICABLE
UPPER FLAMABLE LIMIT	NOT APPLICABLE
LOWER FLAMABLE LIMIT	NOT APPLICABLE
AUTO-IGNITION TEMPERATURE	NOT APPLICABLE
HAZARDOUS COMBUSTION PRODUCTS	BURNING MAY PRODUCE OXIDES OF CARBON NITROGEN
UNUSUAL FIRE HAZARD	AVOID DUSTING

EXPLOSION HAZARD

SENSITIVITY TO STATIC DISCHARGE	REMOTE POSSIBILITY OF A DUST EXPLOSION. IF MIXED WITH AIR IN THE PROPER PROPORTIONS, IT CAN BE EXPLOSIVE (SIMILAR TO FLOUR OR STARCH).
SENSITIVITY TO MECHANICAL IMPACT	NOT APPLICABLE

REACTIVITY DATA

PRODUCT STABILITY	STABLE
PRODUCT INCOMPATIBILITY	AVOID BROMINE TRIFLUORDE, LITHIUM, STRONG ACIDS, BASES AND OXIDIZERS.
CONDITIONS OF REACTIVITY	NONE KNOWN
HAZARDOUS DECOMPOSITION PRODUCTS	BURNING MAY YIELD OXIDES OF CARBON

TOXICOLOGICAL PROPERTIES

SYMPTOMS OF OVER EXPOSURE FOR EACH POTENTIAL ROUTE OF ENTRY:

INHALLATION, ACUTE	MAY CAUSE IRRITATION IF DUST IS INHALED.
INHALATION, CHRONIC	NONE KNOWN
SKIN CONTACT	MAY BE IRRITATING TO THE SKIN. WILL CAUSE YELLOW/GREEN STAINING OF THE SKIN ON CONTACT.
EYE CONTACT	MAY CAUSE IRRITATION
INGESTION	URINE MAY BE A YELLOW/GREEN COLOR UNTIL THE DYE HAS BEEN WASHED THROUGH THE SYSTEM.
EFFECTS OF ACUTE EXPOSURE	DIRECT CONTACT MAY CAUSE IRRITATION TO THE EYES, SKIN, AND RESPIRATORY TRACT.
EFFECTS OF CHRONIC EXPOSURE	NONE KNOWN
THRESHOLD OF LIMIT VALUE	NOT APPLICABLE
CARCINOGENICITY	NOT LISTED AS A KINOWN OR SUSPECTED CARCINOGEN BY IARC, NTP OR OSHA.
TERATOGENICITY	NONE KNOWN
TOXICOLOGY SYNERGISTIC PRODUCTS	NONE KNOWN

PREVENTATIVE MEASURES

PERSONAL PROTECTIVE EQUIPMENT

GLOVES	RUBBER
RESPIRATORY	USE NISOH APPROVED DUST MASK IF DUSTY CONDITIONS EXIST.
CLOTHING	PROTECTIVE CLOTHING SHOULD BE WORN WHERE CONTACT IS UNAVOIDABLE.
OTHER	HAVE ACCESS TO EMERGENCY EYEWASH.

PREVENTATIVE MEASURES (CONT.)

ENGINEERING CONTROLS	NOT NECESSARY UNDER NORMAL CONDITIONS, USE LOCAL VENTILATION IF DUSTY CONDITIONS EXIST.
SPILL OR LEAK RESPONSE	SWEEP UP SPILLS AND PLACE IN WASTE DISPOSAL CONTAINER, FLUSH AFFECTED AREA WITH WATER.
WASTE DISPOSAL	INCINERATE OR REMOVE TO A SUITABLE SOLID WASTE DISPOSAL SITE, DISPOSE OF ALL WASTES IN ACCORDANCE WITH FEDERAL, STATE AND LOCAL REGULATIONS.
HANDELING PROCEDURES AND EQUIPMENT	NO SPECIAL REQUIREMENTS.
STORAGE REQUIREMENTS	STORE IN A COOL, DRY PLACE AWAY FROM EXCESSIVE HEAT OR FLAME.
SHIPPING INFORMATION	NO SPECIAL REQUIREMENTS

FIRST AID MEASURES

FIRST AID EMERGENGY PROCEDURES

EYE CONTACT	FLUSH EYES WITH WATER FOR AT LEAST 15 MINUTES. GET MEDICAL ATTENTION IF IRRITATION PERSISTS.
SKIN CONTACT	WASH SKIN THOROUGHLY WITH SOAP AND WATER. GET MEDICAL ATTENTION IF IRRITATION DEVELOPS.
INHALATION	IF DUST IS INHALED, MOVE TO FRESH AIR. IF BREATHING IS DIFFICULT GIVE OXYGEN AND GET IMMEDIATE MEDICAL ATTENTION.
INGESTION	DRINK PLENTY OF WATER AND INDUCE VOMITING. GET MEDICAL ATTENTION IF LARGE QUANTITIES WERE INGESTED OR IF NAUSEA OCCURS. NEVER GIVE FLUIDS OR INDUCE VOMITING IF THE PERSON IS UNCONSCIOUS OR HAS CONVULSIONS.

SPECIAL NOTICE

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END OF MATERIAL SAFETY DATA SHEET

Division of Kingscote Chemicals

WATER TRACING DYE
FWT RED PRODUCTS

TECHNICAL DATA BULLETIN

Bright Dyes FWT Red products are specially formulated versions of Rhodamine WT dye for convenient use in water tracing and leak detection studies. This bright, fluorescent red dye is certified by NSF International to ANSI/NSF Standard 60 for use in drinking water. It may be detected visually, by ultraviolet light and by appropriate fluorometric equipment. Today it is most often used visually. Visually the dye appears bright pink to red, depending on its concentration and under ultraviolet light as bright orange.

The dye is resistant to absorption on most suspended mater in fresh and salt water. Compared to Bright Dyes FLT Yellow/Green products it is significantly more resistant to degradation by sunlight and when used in fluorometry, stands out much more clearly against background fluorescence. As always the use and suitability of these products for any specific application should be evaluated by a qualified hydrologist or other industry professional.

General Properties	Tablets	FWT Red 25 Liquid	Powders
Detectability of active ingredient [1]	Visual <100 ppb	Visual <100 ppb	Visual <100 ppb
Maximum absorbance wavelength [2]	550/588 nm	550/588 nm	550/588 nm
Appearance	Dark red convex 1.6cm diameter	Clear dark red aqueous solution	Dark red fine powder
NSF (Max use level in potable water)	0.3 ppb	0.8 ppb	0.1 ppb
Weight	1.05 gms ± 0.05		
Dissolution Time [3]	50% < 3 minutes 95% < 6 minutes		50% < 3 minutes 95% < 6 minutes
Specific Gravity		1.03 ± 0.05 @ 25° C	
Viscosity [4]		1.3 cps	
pH		8.7 ± 0.5 @ 25° C	

Coverage of Products	One Tablet	One Pint Liquid	One Pound Powder
Light Visual	604 gallons	31,250 gallons	604,000 gallons
Strong Visual	60 gallons	3,125 gallons	60,400 gallons

Caution: These products may cause irritation and/or staining if allowed to come in contact wit the skin. The use of gloves and goggles is recommended when handling this product, as with any other dye or chemical.

[1] In deionized water in 100 ml flask. Actual detectability and coverage in the field will vary with specific water conditions.
[2] No significant change in fluorescence between 6 and 11 pH.
[3] (One tablet, 1 gram of powder), in flowing deionized water in a 10 gallon tank.
[4] Measured on a Brookfield viscometer, Model LV, UL adapter, 60 rpm @ 25° C.

Kingscote Chemicals, 3334 S. Tech Blvd., Miamisburg, Ohio 45342
Telephone (937) 886-9100 Fax (937)886-9300 Web:www.brightdyes.com

MSDS PREPARATION INFORMATION

PREPARED BY:	T. P. MULDOON (937) 886-9100
DATE PREPARED:	1/1/08

PRODUCT INFORMATION

MAUNFACTURED BY:	KINGSCOTE CHEMICALS 3334 S. TECH BLVD. MIAMISBURG, OHIO 45342
CHEMICAL NAME	MIXTURE
CHEMICAL FORMULA	NOT APPLICABLE
CHEMICAL FAMILY	XANTHENE DYE FORM

HAZARDOUS INGREDIENTS

NONE PER 29 CFR 1910.1200

PHYSICAL DATA

PHYSICAL STATE	DRY POWDER
ODOR AND APPEARANCE	DARK RED COLOR WITH NO APPARENT ODOR
SPECIFIC GRAVITY	>1
VAPOR DENSITY (mm Hg @ 25 ° C)	NOT APPLICABLE
VAPOR DENSITY (AIR =1)	NOT APPLICABLE
EVAPORATION RATE (Butyl Acetate = 1)	NOT APPLICABLE
BOILING POINT	NOT APPLICABLE
FREEZING POINT	NOT APPLICABLE
pH	NOT APPLICABLE
SOLUBILITY IN WATER	HIGHLY SOLUBLE WITH SMALL AMOUNT OF INSOLUBLE RESIDUE

FIRE HAZARD

CONDITION OF FLAMMABILITY	NON-FLAMABLE
MEANS OF EXTINCTION	WATER FOG, CARBON DIOXIDE, OR DRY CHEMICAL
FLASH POINT AND METHOD	NOT APPLICABLE
UPPER FLAMABLE LIMIT	NOT APPLICABLE
LOWER FLAMABLE LIMIT	NOT APPLICABLE
AUTO-IGNITION TEMPERATURE	NOT APPLICABLE
HAZARDOUS COMBUSTION PRODUCTS	BURNING MAY PRODUCE OXIDES OF CARBON NITROGEN
UNUSUAL FIRE HAZARD	REMOTE POSSIBILITY OF A DUST EXPLOSION. IF MIXED WITH AIR IN THE PROPER PROPORTIONS, IT CAN BE EXPLOSIVE (SIMILAR TO FLOUR OR STARCH).

EXPLOSION HAZARD

SENSITIVITY TO STATIC DISCHARGE	REMOTE POSSIBILITY OF A DUST EXPLOSION. IF MIXED WITH AIR IN THE PROPER PROPORTIONS, IT CAN BE EXPLOSIVE (SIMILAR TO FLOUR OR STARCH).
SENSITIVITY TO MECHANICAL IMPACT	NOT APPLICABLE

REACTIVITY DATA

PRODUCT STABILITY	STABLE
PRODUCT INCOMPATIBILITY	AVOID BROMINE TRIFLUORDE, LITHIUM, STRONG ACIDS, BASES AND OXIDIZERS.
CONDITIONS OF REACTIVITY	NONE KNOWN
HAZARDOUS DECOMPOSITION PRODUCTS	SEE HAZARDOUS COMBUSTION PRODUCTS

TOXICOLOGICAL PROPERTIES

SYMPTOMS OF OVER EXPOSURE FOR EACH POTENTIAL ROUTE OF ENTRY:

INHALLATION, ACUTE	MAY CAUSE IRRITATION IF DUST IS INHALED.
INHALATION, CHRONIC	NONE KNOWN
SKIN CONTACT	MAY BE IRRITATING TO THE SKIN. WILL CAUSE STAINING OF THE SKIN ON CONTACT.
EYE CONTACT	MAY CAUSE IRRITATION
INGESTION	URINE MAY BE A YELLOW/GREEN COLOR UNTIL THE DYE HAS BEEN WASHED THROUGH THE SYSTEM.
EFFECTS OF ACUTE EXPOSURE	DIRECT CONTACT MAY CAUSE IRRITATION TO THE EYES, SKIN, AND RESPIRATORY TRACT.
EFFECTS OF CHRONIC EXPOSURE	NONE KNOWN
THRESHOLD OF LIMIT VALUE	NOT APPLICABLE
CARCINOGENICITY	NOT LISTED AS A KINOWN OR SUSPECTED CARCINOGEN BY IARC, NTP OR OSHA.
TERATOGENICITY	NONE KNOWN
TOXICOLOGY SYNERGISTIC PRODUCTS	NONE KNOWN

PREVENTATIVE MEASURES

PERSONAL PROTECTIVE EQUIPMENT

GLOVES	RUBBER
RESPIRATORY	USE NISOH APPROVED DUST MASK IF DUSTY CONDITIONS EXIST.
CLOTHING	PROTECTIVE CLOTHING SHOULD BE WORN WHERE CONTACT IS UNAVOIDABLE.
OTHER	HAVE ACCESS TO EMERGENCY EYEWASH.

PREVENTATIVE MEASURES (CONT.)

ENGINEERING CONTROLS	NOT NECESSARY UNDER NORMAL CONDITIONS, USE LOCAL VENTILATION IF DUSTY CONDITIONS EXIST.
SPILL OR LEAK RESPONSE	SWEEP UP SPILLS AND PLACE IN WASTE DISPOSAL CONTAINER, FLUSH AFFECTED AREA WITH WATER.
WASTE DISPOSAL	INCINERATE OR REMOVE TO A SUITABLE SOLID WASTE DISPOSAL SITE, DISPOSE OF ALL WASTES IN ACCORDANCE WITH FEDERAL, STATE AND LOCAL REGULATIONS.
HANDELING PROCEDURES AND EQUIPMENT	NO SPECIAL REQUIREMENTS.
STORAGE REQUIREMENTS	STORE IN A COOL, DRY PLACE AWAY FROM EXCESSIVE HEAT OR FLAME.
SHIPPING INFORMATION	NO SPECIAL REQUIREMENTS

FIRST AID MEASURES

FIRST AID EMERGENGY PROCEDURES

EYE CONTACT	FLUSH EYES WITH WATER FOR AT LEAST 15 MINUTES. GET MEDICAL ATTENTION IF IRRITATION PERSISTS.
SKIN CONTACT	WASH SKIN THOROUGHLY WITH SOAP AND WATER. GET MEDICAL ATTENTION IF IRRITATION DEVELOPS.
INHALATION	IF DUST IS INHALED, MOVE TO FRESH AIR. IF BREATHING IS DIFFICULT GIVE OXYGEN AND GET IMMEDIATE MEDICAL ATTENTION.
INGESTION	DRINK PLENTY OF WATER AND INDUCE VOMITING. GET MEDICAL ATTENTION IF LARGE QUANTITIES WERE INGESTED OR IF NAUSEA OCCURS. NEVER GIVE FLUIDS OR INDUCE VOMITING IF THE PERSON IS UNCONSCIOUS OR HAS CONVULSIONS.

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END OF MATERIAL SAFETY DATA SHEET

APPENDIX B

Project Forms

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Dye Deliverly Rate Record

Date	Time	Injection Well	Effluent Injection Rate (mgd)	Dye Delivery Rate (gph)	Tracer Concentration (ppb)	Remarks

Appendix B: Form A

TWO-POINT FLUOROMETER ACCURACY CHECK

Instrument Information

Model ______________________ Serial Number: ______________________

Date	Time	Room Temp. (^{o}C)	Conc. of Stand. Value (ppb)	Initial Reading (ppb)	Corrected Reading (ppb)	Operator's initials and remarks

Appendix B: Form B

DAILY FIELD SAMPLING FORM

Date Sample Collected	Time	Sample location	Sample Number	Temp. (^{o}C)	Spec. Cond. (μs/cm)	Salinity ($^{o}/_{oo}$)	Operator's initials and remarks

Appendix B: Form C

FIELD FLUOROMETRY RECORD

Instrument Information

Model ______________________ Serial Number: ______________________

Date	Time	Room Temp. (^{o}C)	Distilled Water Blank Fl. (ppb)	Sample Fl. (ppb)	Operator's initials and remarks

Appendix B: Form D

LABORATORY FLUOROMETRY RECORD

Instrument Information

Model ______________________ Serial Number: ______________________

Date	Time	Room Temp. (^oC)	Distilled Water Blank Fl. (ppb)	Sample Fl. (ppb)	Background Fl. (ppb)	Corrected Fl. (ppb)	Operator's initials and remarks

Lahaina Groundwater Tracer Study
Chain of Custody Form

Ship to:		Sampler Name:	Send Results to:
		Sampler Signature	
Carrier	Air Bill #		

Field Sample ID	Date	Time	Type	Presvtn.	Filt - Unfilt	# of Containters	FS? (Y/N)	Analysis Required				comments

Sample Condition Upon Receipt at Laboratory	
Special Instructions/Comments	

#1 Released by (Sig.)	Date	#2 Released by (Sig.)	Date	#3 Released by (Sig.)	Date
Organization Name:	time	Organization Name:	time	Organization Name:	time
#2 Received by: (Sig.)	Date	#3 Received by: (Sig.)	Date	#4 Released by (Sig.)	Date
Organization Name:	Time	Organization Name:	Time	Organization Name:	time

Appendix B: Form F

FLUOROMETER MULTIPOINT CALIBRATION FORM

Instrument Information

Model ______________________ Serial Number: ____________

Temp. of Lab (^oC)

Pre-Adjustment

Date	Time	Standard Conc. (ppb)	Analyzer Response (ppb)	Difference (response-standard) (ppb)	Comments

Regression Analysis: y=mx+b (where y=analyzer response and x=input conc.)

Slope:	Intercept: (ppm)	Correlation:
(0.900 to 1.100)	(-0.010 to 0.010)	(>=0.9960)

Post-Adjustment

Date	Time	Standard Conc. (ppb)	Analyzer Response (ppb)	Difference (response-standard) (ppb)	Comments

Regression Analysis: y=mx+b (where y=analyzer response and x=input conc.)

Slope:	Intercept: (ppm)	Correlation:
(0.900 to 1.100)	(-0.010 to 0.010)	(>=0.9960)

Appendix B: Form G

WATER QUALITY ANALYZER DAILY ACCURACY/CALIBRATION CHECK

Instrument Information									
Model				Serial Number					
		Parameter							
Date	Time	(Spec. Cond., or pH)	Units	Std. Lot. No.	Exp. Date	Conc. or Stand.	Initial Reading	Corrected Reading	Operator's initials and remarks

Appendix B: Form H

APPENDIX C

Aquafluor Handheld Fluorometer and Turbidity User's Manual

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*Aqua*fluor™

Handheld Fluorometer and Turbidimeter

User's Manual

Dated: September 2004
Version: 1.3
P/N 998-0851

TURNER DESIGNS
Reliable Instruments for an Unreliable World
845 W.Maude Ave.
Sunnyvale, CA 94085
www.turnerdesigns.com
Telephone 408-749-0994
Toll Free 877-316-8049
Fax 408-749-0998

Table of Contents

Appendices:

1. Introduction

1.1 Description

The *Aqua*fluor™ is a lightweight, handheld fluorometer/turbidimeter. Its dual channel capability allows the user to measure either fluorescence or turbidity in one sample.

The Aquafluor can be configured for any two channels as follows: *in vivo* chlorophyll *a*; cyanobacteria (phycocyanin or phycoerythrin); turbidity, Rhodamine WT; fluorescein; ammonium and extracted chlorophyll a.

1.2 Inspection and Setup

1.2.1 Inspection

Upon receiving your instrument, please inspect everything carefully and make sure all accessories are present. All shipments include:

- The *Aqua*fluor™
- The User's Manual
- 4 AAA batteries
- Cuvettes – Qty 4, See Appendix A5
- Storage Pouch

1.2.2 Setup

Before the *Aqua*fluor can be used, the supplied batteries must be installed.

1. On the backside of the instrument, remove the battery panel by loosening the center screw and then gently pull on the screw. There is some resistance due to the o-ring, used for watertight sealing (see Section 2 for diagram).

2. Install the 4 AAA batteries and verify the batteries are positioned correctly.
3. Replace the battery panel and press it down into position.
4. Hold the battery panel down in place and gently tighten the screw until it is snug. Do not over tighten the screw.

Note: If the battery panel is very difficult to press down or remove, you can apply a small amount of silicon based o-ring grease to lubricate the o-ring as needed.

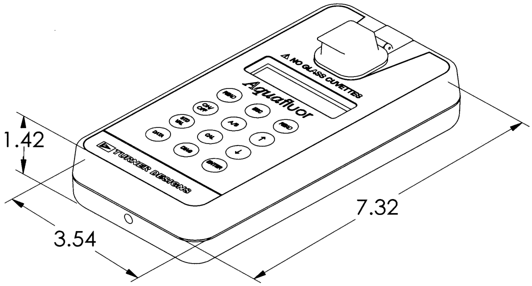
1.3 General Information, Precautions and Cleaning

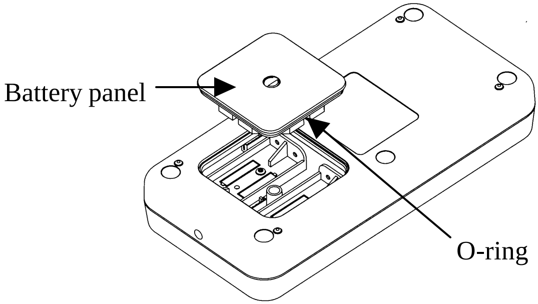
- The sample compartment cannot accept 10mm square glass or quartz cuvettes because their cuvette wall thickness exceeds the 12 mm maximum outer dimension that the *Aqua*fluor can accept. The 10 mm size is the internal dimension of the cuvette and some plastic cuvettes may also be too large.
- Do not force oversized cuvettes into the sample compartment. This can damage the sample compartment. If the cuvette does not easily fit down inside the *Aqua*fluor you will need to purchase different cuvettes.
- Round glass test tubes can be used with an optional adapter that is available. Refer to Appendix A5 for cuvette information.
- Use caution around solvents because they may attack the plastic case of the *Aqua*fluor.
- If a sample is accidentally spilled inside the Sample Compartment, you can invert the *Aqua*fluor to drain out the excess liquid. Then wipe the inside area dry with a clean soft towel or tissue.
- If extra cleaning is needed, use a mild detergent to dampen the towel for cleaning.

- Do not submerge the *Aqua*fluor in water.

- Do not expose the *Aqua*fluor to temperatures outside the specified range of 5 to 40 ^{o}C, or damage may occur to the unit that will not be covered under warranty.

2. Quick View Diagrams





⚠ NO GLASS CUVETTES
Aquafluor
READ
ESC
READ
ON/
OFF
A/B
↑
STD
VAL
CAL
↓
DATA
DIAG
ENTER
TURNER DESIGNS

3. Instrument Operation and Calibration

3.1 Instrument Power Up

To turn on the *Aqua*fluor, press the <ON/OFF> button. After a 5 second warm up, the *Aqua*fluor is ready for operation.
Pressing the <ON/OFF> button again will turn the unit off or if left idle for 3 minutes the unit will turn itself off to save battery power.

A set of new batteries will last for over 1,000 sample readings. If the batteries have low power or are not positioned properly, the following warning message will be displayed –
“Batt Level < 20% !! Caution !!”

The contrast of the display can change with temperature and will get lighter as the unit gets colder. The contrast can be easily adjusted. Press the ↑ arrow button to darken the contrast and press the ↓ arrow to lighten the display contrast.

3.2 Detection Channel Configuration

The *Aqua*fluor has 2 detection channels that are configured as two fluorescent channels or one fluorescent and one turbidity channel.
You should identify the configuration by looking at the label on the back side of the *Aqua*fluor. Refer to the table in Appendix A4 to confirm the correct configuration for your application.

The appropriate channel is selected by pressing the <A/B> button to toggle between the 2 channels. The display will show a label in the lower left corner of the Home screen to identify which channel is activated.

3.3 Calibration Overview

The *Aqua*fluor can be calibrated using Primary or Secondary standards. A Primary Standard is one that contains the same fluorescent material that you are measuring in your unknown samples. The *Aqua*fluor will give an actual quantitative concentration reading when a primary standard of known concentration is used for calibration. The standard and samples must be in the linear detection range to get accurate quantitative results. Refer to Section 4.3 for more details about the linear range and quenching of the samples.

For some applications, Secondary Standards are used for calibration. A secondary standard is one that contains a different type of fluorescent material than your samples. The *in vivo* Chlorophyll application is an example where a Secondary Standard is often used for the calibration, and we recommend using the Solid Secondary Standard (PN 8000-950). Refer to Appendix C for details on Chlorophyll applications and calibration. This appendix also has details about the Ammonium and Turbidity applications.

When a Secondary Standard is used for calibration, the *Aqua*fluor will give relative sample readings that are proportional to the measured fluorescence. In some cases these relative sample readings will be correlated back to actual concentrations that are determined later. For example, this is commonly done for *in vivo* Chlorophyll monitoring applications as described in Appendix C1.

For Dye tracing applications the calibration is normally performed with a primary standard made from the same dye that is being used for the testing. The primary standard will either be made to a known concentration, typically in ppb or ug/L units, or to a known dilution factor. Ideally the Primary Standard and Blank samples used for calibrating will be made with the same water the tests are being performed in. For more details on this and tracer dye use, please refer to the Application Note, "A Practical Guide to Flow Measurement" at the following web link: http://www.turnerdesigns.com/t2/doc/appnotes

3.4 Calibration procedure

It is recommended for best accuracy, that you always calibrate before performing your sample analysis. The *Aqua*fluor will save the calibration settings for each channel until a new calibration is performed.

If the temperature of your samples or the *Aqua*fluor changes significantly, the readings may show a small shift and in this case, you should consider recalibrating. The solid secondary standard is useful for checking the reading stability over time and can also be used to recalibrate if needed.

The *Aqua*fluor is designed with "ambient light rejection". The black sample compartment cover does NOT need to be closed when calibrating or reading samples. This allows for the use of cuvettes with different height dimensions, such as the 12x75 mm round glass tubes.

The orientation and cleanliness of the cuvettes can have an impact on the accuracy of your results.

Refer to Section 4, Sample Analysis Guidelines for information to help insure the best results for your analysis.

3.4.1 Assign a Calibration Standard Value.

This defines the numeric value that you want the standard to read. For example, if you calibrate with a primary standard that has a concentration of 50 ug/L, then you will set the value to 50 and the implied unit of measure will be in ug/L. If you use a Secondary standard to calibrate with, you will set this value to a desired relative value and call the units RFU (Relative Fluorescent Units).

1. Press the <STD VAL> button.
2. Use the ↑ and ↓ arrow buttons to set the standard value. Holding down either arrow button down will allow you to change the value using fast scrolling.
3. When finished, Press the <ENT> or <ESC> button to accept the value and to return to the Home screen.

3.4.2 Perform the Calibration.

1. Press the <CAL> button.
2. Press <ENT> to start the calibration.
3. Insert your blank sample and press <ENT>. The *Aqua*fluor will average the reading for 10 seconds and set the blanking zero point.
4. Insert the standard sample and press <ENT>. The reading is averaged for 10 seconds and the Standard Calibration value is set.
5. Press <ENT> when the calibration is complete to accept the calibration. If <ENT> is not pressed within 10 seconds, you will be asked if you want to abort the calibration.

Press the ↑ or ↓ arrow button to abort or accept the calibration respectively.

If at anytime during steps 1-4 you want to stop the calibration, press <ESC>. This will return you to the Home screen and will default the instrument to the previous calibration.

3.5 Instructions for using the Adjustable Secondary Standard.

1. Calibrate the Aquafluor with a solution of known concentration.
2. Take the adjustable secondary standard from its storage bag. Using the 0.05 allen wrench provided, loosen the locking screw on the back of the secondary standard by turning it counterclockwise one turn. (See photo 1)

Photo 1. Loosening the locking setscrew on the backside of the adjustable secondary standard

3. Place the adjustable secondary standard in the fluorometer sample compartment with the handle towards the rear of the instrument. (See photo 2).

Photo 2. Placing the adjustable secondary standard in the Aquafluor

4. Close the lid
5. Wait 15 seconds, then read the value.
6. Record the value of the secondary standard.
7. Open the lid, and use the 3/32” allen wrench provided to adjust the attenuation screw through the hole at the top of the secondary standard to increase or decrease the value displayed on the screen. (Turning the screw counterclockwise will increase the reading). (See Photo 3)

Photo 3. Adjusting the value on the secondary standard by turning the attenuation screw.

8. Repeat steps 3 – 6 until the secondary standard reads the concentration value of interest. Record the secondary standard reading for future reference.
9. At this point, remove the solid standard and turn the locking setscrew clockwise until it just makes contact with the attenuation screw - **DO NOT OVERTIGHTEN**.
10. Now you can recalibrate the Aquafluor using the value of the secondary standard obtained in step 8.
11. Proceed with analyzing your samples
12. You may use your adjustable secondary standard at any time to check the stability of the fluorometer. Simply insert your standard to read the value. The value should be similar to what was previously obtained in step 8 above.

13. When the secondary standard is not in use, store it in its protective bag to prevent dust collecting on the optical fiber.

On a daily basis, before making measurements with the Aquafluor, use the secondary standard to verify the Aquafluor calibration. If the secondary standard value has changed by more than +/-10% of its assigned value, then the Aquafluor should be recalibrated using the Secondary Standard.

3.6 Sample Analysis

1. Insert your sample. The orientation and cleanliness of the cuvettes can have an impact on the accuracy of your results. Refer to Section 4 for more details.
2. Press either <READ> button. The instrument will measure and average the fluorescence signal for 5 seconds.
3. The reading result will be displayed on the top line of the Home screen.
4. The top left corner will then display “WAIT” for 5 seconds. Once “WAIT” disappears, another sample reading can be performed.

3.7 Diagnostic Information

1. Press <DIAG> to access the diagnostic screens.
2. The first screen shows the number of data points available for internal data logging.
3. Press <ENT> to toggle to the %FS (Full Scale) values for the blank (Blk) and standard (STD) calibration points. The STD value should be at least 3 times larger than the Blk value to insure consistent results. This is often referred to as the Standard to Blank Ratio.

4. Press <ESC> when finished to return to the Home screen.

3.8 Internal Data Logging (IDL)

The Internal Data Logging option allows the downloading of stored data from the *Aqua*fluor. For further information, see Appendix B.

The Aquafluor can log up to 1000 data points. The DATA screens control logging, downloading and erasing the data as described below.

3.8.1 Activate Data Logging

1. Press the <DATA> button 2 times.
2. Press <ENT> to toggle between logging and stop status.
3. Press <ESC> when finished to return to the Home screen.

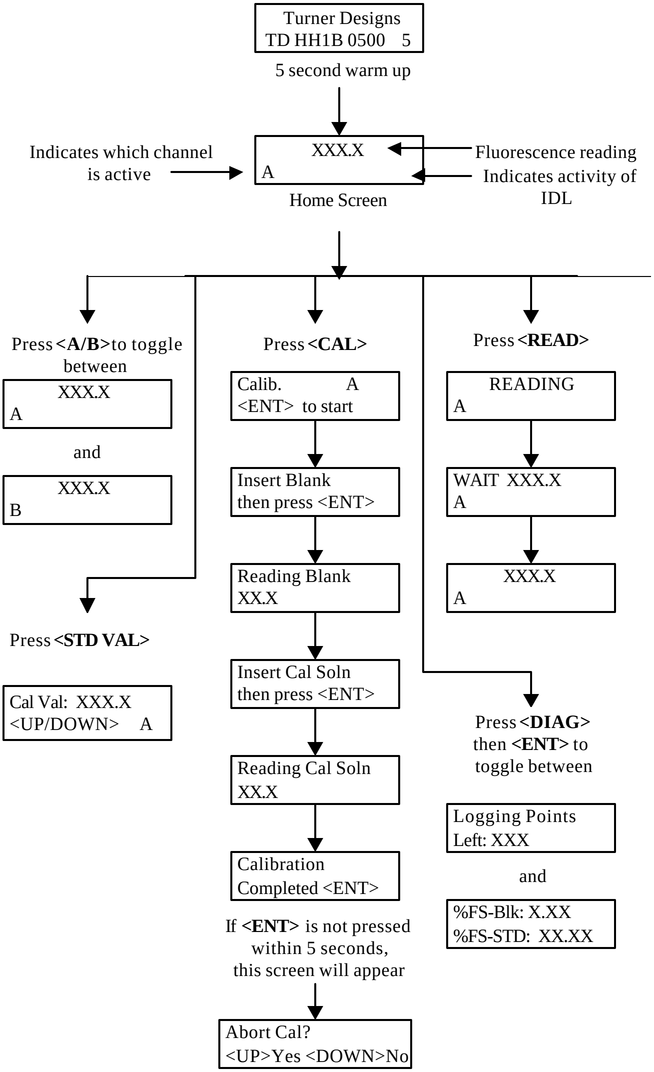
3.8.2 Download Data

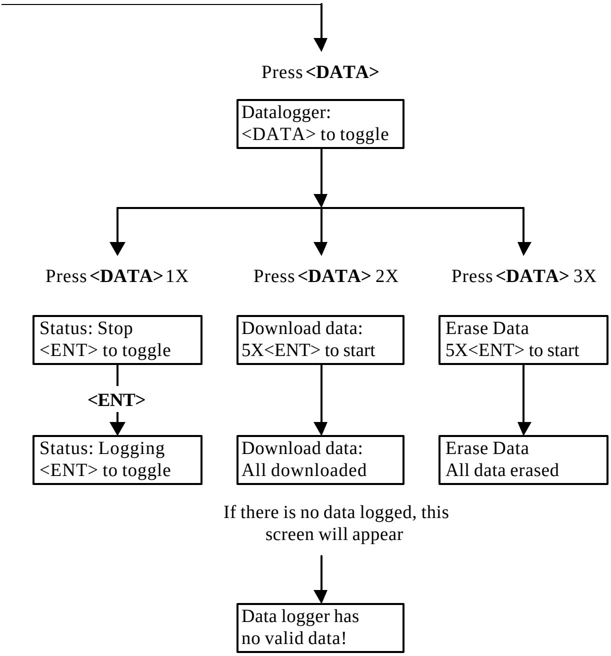
1. Connect the *Aqua*fluor™ to the serial port of your computer.
2. Open the Turner Designs Interface Software. See Appendix B for computer requirements and installation.
3. Press the <DATA> button 3 times.
4. Press <ENT> 5 times to start the data download.
5. Press <ESC> when finished to return to the Home screen.

3.8.3 Erase Data

1. Press the <DATA> button 4 times.
2. Press <ENT> 5 times to erase all logged data.
3. Press <ESC> when finished to return to the Home screen.

3.8 Display Screen Flowchart





Press **<DATA>**
Datalogger:
<DATA> to toggle
Press **<DATA>** 1X
Press **<DATA>** 2X
Press **<DATA>** 3X
Status: Stop
<ENT> to toggle
<ENT>
Status: Logging
<ENT> to toggle
Download data:
5X<ENT> to start
Download data:
All downloaded
If there is no data logged, this
screen will appear
Data logger has
no valid data!
Erase Data
5X<ENT> to start
Erase Data
All data erased

4. Sample Analysis Guidelines

4.1 Handling Samples

1. Take care not to spill samples into the sample chamber. Wipe up any spills promptly.

2. The cuvette MUST BE DRY on the outside when taking readings. Any Moisture or condensation on the outside of the cuvette can effect the reading.

3. Fill the cuvette with at least 2mL solution volume or at least 50% full. Significant error in the readings can result if the cuvette contains less than this minimum volume.

4. The *Aqua*fluor is very sensitive and even small amounts of material from a previous sample may contaminate the sample and result in errors. Use a clean cuvette for all readings. If you are using the same cuvette for your samples it is very important that you thoroughly clean the cuvette between samples. A good way to confirm the cuvette cleanliness is to read a blank solution. If the reading is higher than the normal blank reading, the cuvette is not clean.

5. Any bubbles in the sample will effect the readings. Take care not to introduce bubbles into samples. Remove any bubbles by lightly tapping with your finger on the outside cuvette wall or cover the top of the cuvette and tilt the sample to help dissipate bubbles.

4.2 Positioning Samples

The orientation of the cuvette in the sample compartment can give slightly different readings especially for low concentration samples. This is due to variations in the walls of the cuvette that are not readily visible to the eye. We recommend that the cuvette be marked at the top on one side and positioned in the sample compartment the same way each time for best results.

Turbidity sample measurements are particularly sensitive to the quality and cleanliness of the cuvette. Small scratches or smudges on the cuvette will effect the accuracy of the readings. The Polystyrene cuvettes (P/N 7000-957) give the best Turbidity measurement results, due to better quality of the cuvette.

4.3 Linear Range and Quenching

The linear range is the concentration range in which the readout of the *Aqua*fluor is directly proportional to the concentration of the fluorophore in the sample. The linear range begins with the lowest detectable concentration and spans to an upper limit concentration that is dependent on the properties of the fluorescent material and the cuvette optical path length. For Rhodamine, Fluorescein dye or Chlorophyll in the 10 mm square cuvette, the upper limit of linearity is approximately 300 ppb (ug/L).

At concentrations above this upper limit, the fluorescence reading will not increase at a linear rate in comparison to the change in concentration. At concentrations 10 times higher than the upper limit , the readings will start to decrease even though the sample concentration is increasing.

This effect is known as "sample quenching" and is due to light absorption losses in the sample. See the Figure 1 graph below.

When you start to see visual color in the sample, this is an indicator that the sample may be above the upper limit of linearity. The linearity can be verified by diluting a sample 1:1 or another convenient dilution ratio. If the sample is within the linear range, the reading will decrease in direct proportion to the dilution. If the reading does not decrease in direct proportion to the dilution or if the reading increases, the original sample concentration was above the linear range.

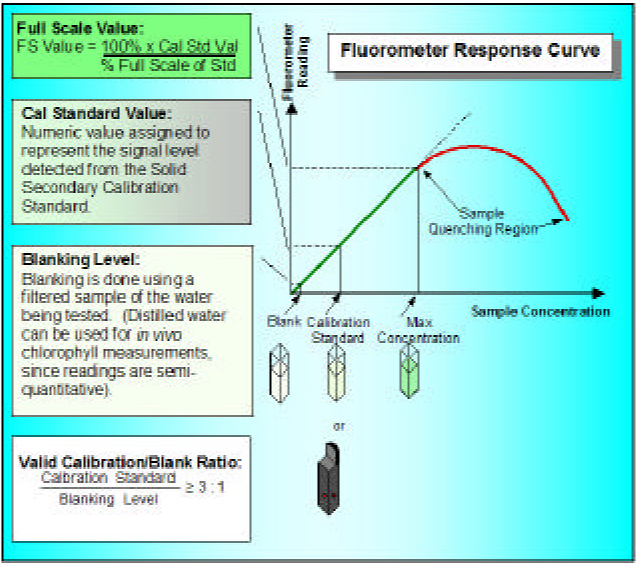


Figure 1. Linearity and Calibration Response Curve

4.4 Temperature Considerations

Fluorescence is temperature sensitive. As the temperature of the sample increases, the fluorescence decreases. For best accuracy make sure your samples are at the same temperature as the calibration standard. Some temperature coefficients examples are: Rhodamine dye is approx. 2.6% per degree C and Fluorescein dye is 0.36% per degree C.

4.5 Data Quality

The *Aqua*fluor is only as accurate as the standards that are used to calibrate it. This is why it is important to take care when preparing standards, samples, and blank. One should follow good laboratory practices when preparing all solutions and samples.

5 Warranty

5.1 Terms

Turner Designs warrants the *Aqua*fluor Fluorometer and accessories to be free from defects in materials and workmanship under normal use and service for a period of one year from the time of initial purchase, with the following restrictions:

The instrument and accessories must be installed, powered, and operated in compliance with the directions in this *Aqua*fluor User's Manual and directions accompanying the accessories.

Damage resulting from measurement of samples found to be incompatible with the materials used in the sample system or resulting from contact with corrosive materials or atmosphere is not covered.

Damage caused by modification of the instrument by the customer is not covered.

Damage incurred in shipping is not covered.

5.2 Warranty Service

To obtain service during the warranty period, the owner shall take the following steps:

1. Write or call the Turner Designs Technical Support and describe as precisely as possible the symptoms or nature of the problem.

Phone: (408) 749-0994
Email: support@turnerdesigns.com

2. Carry out any adjustments or tests as suggested by Technical Support.

3. If proper performance is not obtained, a Return Authorization number (RMA) will be issued to you. Ship the instrument, prepaid, to Turner Designs, with the RMA number referenced and include your complete shipping address and phone number inside.

The instrument will be repaired at no charge, but for customers outside of the United States, any shipment or documentation charges will be billed at cost.

NOTE: The instrument or accessories should not be returned without first contacting Turner Designs. Prior correspondence is needed to understand the nature of the problem and to help decide on the best action to resolve the issue as quickly as possible.

5.3 Out-of-Warranty Service

Proceed exactly as for Warranty Service, above. If our Technical Support can assist you by phone or correspondence, we will be glad to, at no charge.

Repair service will be billed on a fixed price basis. Shipment to Turner Designs should be prepaid. Your bill will include return shipment freight charges.

Address for Shipment:
Turner Designs
845 W. Maude Ave.
Sunnyvale, CA 94085

Appendix A: Instrument Specifications

A1. General Specifications

Specification	**Description**
Size	1.75” x 3.5” x 7.25” (4.45cm x 8.9cm x 18.4cm)
Weight	13.9oz (0.4kg)
Dynamic range	3 orders of magnitude
Resolution	12 bits
LCD Display	2 x 16 characters
Case	Meets IP 67 Standard; dustproof and waterproof
Temperature	41-104 ^{o}F; 5-40 ^{o}C
Detector	Photodiode
Calibration Type	Single-point and blank
Error message	Low battery, High blank
Cuvette Type	See Appendix A5.
Warm Up Time	5 seconds
Auto Power Off	After 3 minutes of inactivity

A2. Optical Configurations for *in vivo* Chlorophyll, Rhodamine and Turbidity

	in vivo Chlor Channel	Rhodamine Channel	Turbidity Channel
Light Source	Blue LED	Green LED	Green LED
Excitation Optics	460±20nm	540±20nm	515±10nm
Emission Optics	>665nm	>570nm	515±10nm
Limit of Detection	0.25ug/l *in vivo* Chl	0.4ppb	0.5NTU
Max range	> 300 ppb	>300ppb	>150 NTU
Temperature coefficients	1.4%/^{o}C Linear	0.026/^{o}C Exponential	N/A

A3. Optical Configurations for Ammonium and Extracted Chlorophyll

	Ammonium (NH4) UV Channel	Extracted Chlorophyll Channel
Light Source	UV LED	Blue LED
Excitation Optics	375nm	430nm
Emission Optics	>420nm	>665nm
Limit of Detection	0.1mM	0.5mg/L
Max range	50 mM	300 mg/L
Temperature coefficients	N/A	0.3/°C Linear

A4. Label Designations for Optical Configurations

Label	**Ex. / Em. nm**	**Applications**
INV CHLOR	460 / >665	*in vivo* Chlor
EXT CHLOR	430 / >665	Extracted Chlor
PC	590 / 660	Phycocyanin
PE	525 / 575	Phycoerythrin
BLU-515	475 / 515	Fluorescein
Green-570	540 / >570	Rhodamine
TURB	515 / 515	Turbidity
UV-405	375 / 405	
UV-420	375 / >420	Ammonium
UV-445	375 / 445	
UV-480	375 / 480	

A5. Cuvette information

Cuvette type	**Size**	**Usage**	**Part No.**
Methacrylate plastic	10 mm Square (height 44.5 mm)	Required for UV (< 400 nm), works for all applications, Do NOT use with solvents (ie. Acetone)	7000-959
Polystyrene Plastic	10 mm Square (height 47.5 mm)	Preferred for Turbidity use, will NOT work for UV, Do NOT use with solvents (ie. Acetone)	7000-957
Borosilicate glass	12 x 75mm Round	Required for Extracted Chlor, where solvents are used. Requires Adapter PN 8000-932	10-029A

A6.Optical Configuration for Cyanobacteria

	Phycocyanin	Phycoerythrin
Light Source	Yellow LED	Green LED
Excitation Optics	595 nm	528 nm
Emission Optics	670 nm	573 nm
Limit of Detection	150 cells/mL	150 cells/mL
Max Range	150,000 cells/mL	150,000 cells/mL
Temperature Coefficients	Not available	Not available

Appendix B: Internal Data Logging

B1. Shipping Checklist

The Internal Data Logging kit (PN 8000-920) contains the following items:

- Interface cable
- Turner Designs Spreadsheet Interface Software on a CD disk.

Both of these items are necessary for downloading or transferring data from the Aquafluor to a PC.

B2. Hardware Requirements

- PC with Windows 95 or later
- MS Excel 5.0 or later
- At least 1 available serial port

B3. Software Installation

1. Exit out of any programs that are running.
2. Insert the CD Disk and select the setup.exe file.
3. Follow the steps in the setup wizard to install the necessary files.
4. When the setup is complete, an icon named “Spreadsheet Interface Software” will be found on the desktop and in the “Programs” menu list.

B4. Connecting

1. Using the cable provided, connect the 9 pin adapter end of the cable into the available serial port of your computer.
2. Plug the opposite end of the cable into the port at the base of the *Aqua*fluor.
3. Open the Spreadsheet Interface software.

4. Click on the box to the right of the COM port icon to select the appropriate COM port. This is typically COM port 1 or 2.
5. Click on "Start" and the program will open an Excel spreadsheet for the data transfer. The indicator boxes to the left should both be green to confirm a good interface connection.
6. Follow the directions from Section 3.7 for collecting and downloading data from the *Aqua*fluor. Data will appear in the Excel spreadsheet. BE SURE to save this data, by performing a "Save File as", after the download has completed.

B5. Real Time Data Transfer

Data can also be transferred directly to the computer after each reading. To do so:

1. Follow steps 1-6 of Section B4 to create the connection between the *Aqua*fluor and your computer.
2. Insert a sample and press the <READ> button. When the reading is finished, the results will automatically transfer to the active Excel spreadsheet.

B6. IDL Troubleshooting

Difficulties can arise when parameters are set incorrectly or improper cable connections. Here are some common solutions.

1. Box to the left of the COM port is red. This means that the COM port is not available. Causes:
   a. Another software program (such as palm pilot/ hot sync) could be using the COM port, making it unavailable. Make sure to

close all programs of this type before opening the Spreadsheet Interface software.

b. The port selected is incorrect. Follow step 4 in section B4 above, to choose the correct COM port.

2. All lights are green, but no data transferred, even though the instrument says “All data downloaded”.

   a. The connection between the instrument and the computer is bad. Check and tighten the cable connections. Make sure both ends of the cable are plugged in tightly.

Appendix C1: *In Vivo* Chlorophyll

In vivo chlorophyll analysis is the fluorescent detection of chlorophyll *a* in algal cells in water. In this technique, the excitation light from the fluorometer passes through the untreated sample of water and causes the chlorophyll *a* within the cells to fluoresce. Environmental conditions, presence of interfering compounds, cellular physiology, morphology, and light history can influence the relationship between the *in vivo* fluorescence and the actual concentration of chlorophyll *a* in the sample. These factors cause *in vivo* fluorescence to be a semi-quantitative tool. Despite its semi-quantitative nature, *in vivo* fluorescence data can supply valuable information on the spatial and temporal distribution of chlorophyll concentrations quickly and easily.

To obtain quantitative data, the *in vivo* fluorescence data must be correlated with extracted chlorophyll *a* data that can be obtained through the extraction and measurement of the pigment from grab samples on a laboratory fluorometer, spectrophotometer or HPLC.

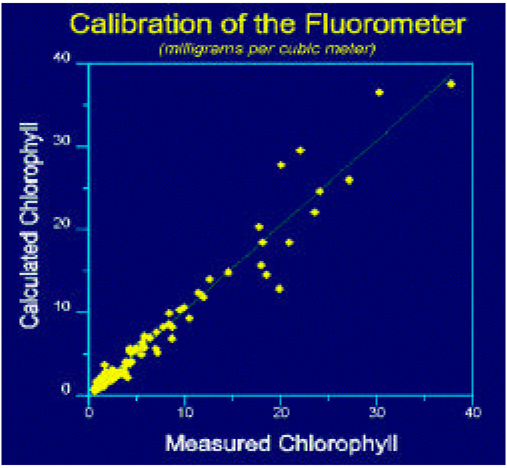
When collecting "grab" samples for chlorophyll extraction, the *in vivo* reading must be noted at the same time the sample is collected. Several samples should be collected within each niche or environment.

Once the chlorophyll concentration has been determined through extraction, the concentration should be correlated with the corresponding *in vivo* value as shown in Graph C1 below.

We recommend using the Solid Secondary Standard to calibrate the Aquafluor for *in vivo* chlorophyll use.

The Solid Standard simulates the *in vivo* fluorescence of a 10 µg/L marine diatom culture. This should be viewed only as a “ballpark” estimate of actual chlorophyll concentration. For this reason, if you assign the Cal Standard Value = 10, this will give relative reading values that will be proportional to the actual chlorophyll concentration in the water sample.

The Aqaufluor calibration also requires a Blank sample and the best “true blank” is the natural water that has been filtered through a GF/F or membrane filter in order to remove the algal cells but to still retain any dissolved components. However, in most cases distilled water is used for the Blank sample since the *in vivo* readings are semi-quantitative.



Graph C1

For more details on chlorophyll analysis, visit Turner Design’s webpage at this link.
http://www.turnerdesigns.com/t2/esupport/home.html

Appendix C2: Extracted Chlorophyll a

In extractive analysis, fluorometric measurements are made on solvent extracts from algal cells to determine quantitative concentrations of chlorophyll and pheophytin. Acetone, methanol, or DMSO can be used as the extraction solvent. A Primary Chlor a standard is used for calibration and the measurements are made using the 12 x 75 mm glass test tubes.

The EPA Method 445 is a popular Chlorophyll extractive method that was developed using a Turner Designs Model 10 fluorometer and is published by the United States Environmental Protection Agency.

The extracted Chlorophyll channel on the *Aqua*fluor requires performing the acidification step to correct for the pheophytin. This is referred to as the “corrected chlor a” method in section 12.2 of the 445 method. Also, in section 10.1 of the EPA 445 method, the calculation for the F_s is not required, because it will always be equal to 1 when using the *Aqua*fluor.

The EPA 445 method is available at the following link.

http://www.epa.gov/nerlcwww/ordmeth.htm

Appendix C3: Ammonium Detection

Accurate determination of ammonium in aquatic environments is a critical measurement when investigating Nitrogen cycling and nutrient dynamics. Historically, methods for ammonium determination have been a source of frustration within the scientific community due to the lack of a simple, accurate and affordable method, particularly for measurements in the submicromolar range.

The new ammonium technique offers researchers and technicians an excellent alternative to the existing colormetric ideophenol blue method. Benefits of the fluorometric method include:

- Sensitivity: Detection in the submicromolar range.
- Accuracy: More accurate than previous methods for low ammonium concentration samples.
- Simple: Requires only one mixed, non-toxic reagent and no special equipment other than a fluorometer.
- Non-toxic Reagents: OPA, sodium sulfite, and sodium borate.

The colometric ideophenol blue method is susceptible to inconsistent results, particularly with submicromolar ammonium concentrations. Whereas, the new fluorometric technique has been proven to provide accurate and precise data over a wide range of water quality, ammonium concentration and salinity. This method is particularly useful for work in oligotrophic systems, where natural ammonium concentrations are commonly in the submicromolar range.

The following fluorometric method is available at the web link below:

Holmes, R.M., A. Aminot, R. Kerouel, B.A. Hooker, B.J. Peterson. 1999. A simple and precise method for measuring ammonium in marine and freshwater ecosystems. Can. J. Fish. Aquat. Sci. 56: 1801-1802

http://www.turnerdesigns.com/t2/doc/appnotes/pdf/ammonium.pdf

Appendix C4: Turbidity

The Turbidity channel has a detection range of 0.5 to 150 NTU. A Primary Turbidity Standard is required for calibration. We recommend using a Turbidity standard in the range of 10 to 100 NTU. The Polystyrene cuvettes are preferred for best turbidity results. Turbidity standards can be purchased from APS Analytical Stds, a division of GFS Chemicals. When selecting these AMCO Standards, select the type for use with “non-ratio” instruments.
For more details refer to the following web link:
http://www.amcoclear.com/amco/index.php

Appendix C5: Fluorescent Tracer Dyes

Fluorescent tracer dyes provide an accurate, cost effective method for measuring water flow levels, mixing zones, time of travel, groundwater transport, leak detection, retention times, etc. The two most commonly used dyes tracers are Fluorescein and Rhodamine WT. The Fluorescein dye can be detected using the Blue channel on the Aquafluor and Rhodamine dye is detected using the Green channel. The linear detection range for both dyes is 0.4 to 300 PPB (active ingredient) in potable water.

For more information on tracer dye use, refer to the Application Note section at the following web link:

http://www.turnerdesigns.com/t2/doc/appnotes/main.html

Appendix C6: Cyanobacteria Monitoring

The Cyanobacteria channel of the Aquafluor continually senses the concentration of either phycocyanin (PC) or phycoerythrin (PE) fluorescent pigments unique to Cyanobacteria.

Phycobilin pigments are a group of accessory pigments unique to Cyanobacteria. PC and PE are two phycobilin pigments that also happen to have strong and unique fluorescent signals that can be detected by the Cyanobacteria channel.

The fluorescence is measured directly using in vivo cyanobacteria detection, without extraction or chemical treatment. For many types of qualitative work, *in vivo* measurements alone may provide sufficient information. For quantitative measurements, the in vivo data is calibrated by correlation with other measurements, such as cell counts or extracted pigment analysis.

APPENDIX D

Method to Determine Limit of Detection

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Home Page > Executive Branch > Code of Federal Regulations > Electronic Code of Federal Regulations

Electronic Code of Federal Regulations
e-CFR TM

e-CFR Data is current as of June 1, 2011

Title 40: Protection of Environment

PART 136—GUIDELINES ESTABLISHING TEST PROCEDURES FOR THE ANALYSIS OF POLLUTANTS

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Appendix B to Part 136—Definition and Procedure for the Determination of the Method Detection Limit—Revision 1.11

Definition

The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the analyte.

Scope and Application

This procedure is designed for applicability to a wide variety of sample types ranging from reagent (blank) water containing analyte to wastewater containing analyte. The MDL for an analytical procedure may vary as a function of sample type. The procedure requires a complete, specific, and well defined analytical method. It is essential that all sample processing steps of the analytical method be included in the determination of the method detection limit.

The MDL obtained by this procedure is used to judge the significance of a single measurement of a future sample.

The MDL procedure was designed for applicability to a broad variety of physical and chemical methods. To accomplish this, the procedure was made device- or instrument-independent.

Procedure

1. Make an estimate of the detection limit using one of the following:

(a) The concentration value that corresponds to an instrument signal/noise in the range of 2.5 to 5.

(b) The concentration equivalent of three times the standard deviation of replicate instrumental measurements of the analyte in reagent water.

(c) That region of the standard curve where there is a significant change in sensitivity, *i.e.* , a break in the slope of the standard curve.

(d) Instrumental limitations.

It is recognized that the experience of the analyst is important to this process. However, the analyst must include the above considerations in the initial estimate of the detection limit.

2. Prepare reagent (blank) water that is as free of analyte as possible. Reagent or interference free

water is defined as a water sample in which analyte and interferent concentrations are not detected at the method detection limit of each analyte of interest. Interferences are defined as systematic errors in the measured analytical signal of an established procedure caused by the presence of interfering species (interferent). The interferent concentration is presupposed to be normally distributed in representative samples of a given matrix.

3. (a) If the MDL is to be determined in reagent (blank) water, prepare a laboratory standard (analyte in reagent water) at a concentration which is at least equal to or in the same concentration range as the estimated method detection limit. (Recommend between 1 and 5 times the estimated method detection limit.) Proceed to Step 4.

(b) If the MDL is to be determined in another sample matrix, analyze the sample. If the measured level of the analyte is in the recommended range of one to five times the estimated detection limit, proceed to Step 4.

If the measured level of analyte is less than the estimated detection limit, add a known amount of analyte to bring the level of analyte between one and five times the estimated detection limit.

If the measured level of analyte is greater than five times the estimated detection limit, there are two options.

(1) Obtain another sample with a lower level of analyte in the same matrix if possible.

(2) The sample may be used as is for determining the method detection limit if the analyte level does not exceed 10 times the MDL of the analyte in reagent water. The variance of the analytical method changes as the analyte concentration increases from the MDL, hence the MDL determined under these circumstances may not truly reflect method variance at lower analyte concentrations.

4. (a) Take a minimum of seven aliquots of the sample to be used to calculate the method detection limit and process each through the entire analytical method. Make all computations according to the defined method with final results in the method reporting units. If a blank measurement is required to calculate the measured level of analyte, obtain a separate blank measurement for each sample aliquot analyzed. The average blank measurement is subtracted from the respective sample measurements.

(b) It may be economically and technically desirable to evaluate the estimated method detection limit before proceeding with 4a. This will: (1) Prevent repeating this entire procedure when the costs of analyses are high and (2) insure that the procedure is being conducted at the correct concentration. It is quite possible that an inflated MDL will be calculated from data obtained at many times the real MDL even though the level of analyte is less than five times the calculated method detection limit. To insure that the estimate of the method detection limit is a good estimate, it is necessary to determine that a lower concentration of analyte will not result in a significantly lower method detection limit. Take two aliquots of the sample to be used to calculate the method detection limit and process each through the entire method, including blank measurements as described above in 4a. Evaluate these data:

(1) If these measurements indicate the sample is in desirable range for determination of the MDL, take five additional aliquots and proceed. Use all seven measurements for calculation of the MDL.

(2) If these measurements indicate the sample is not in correct range, reestimate the MDL, obtain new sample as in 3 and repeat either 4a or 4b.

5. Calculate the variance (S^2) and standard deviation (S) of the replicate measurements, as follows:

$$S^2 = \frac{1}{n-1}\left[\sum_{i=1}^{n} x_i^2 - \frac{\left(\sum_{i=1}^{n} X_i\right)^2}{n}\right] S = \left(S^2\right)^{\frac{1}{2}}$$

where:

X_i; i=1 to n, are the analytical results in the final method reporting units obtained from the n sample aliquots and Σ refers to the sum of the X values from i=l to n.

6. (a) Compute the MDL as follows:

MDL = t(n-1,1-α=0.99) (S)

where:

MDL = the method detection limit

t(n-1,1-α=.99)= the students' t value appropriate for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom. See Table.

S = standard deviation of the replicate analyses.

(b) The 95% confidence interval estimates for the MDL derived in 6a are computed according to the following equations derived from percentiles of the chi square over degrees of freedom distribution (χ^2 /df).

LCL = 0.64 MDL

UCL = 2.20 MDL

where: LCL and UCL are the lower and upper 95% confidence limits respectively based on seven aliquots.

7. Optional iterative procedure to verify the reasonableness of the estimate of the MDL and subsequent MDL determinations.

(a) If this is the initial attempt to compute MDL based on the estimate of MDL formulated in Step 1, take the MDL as calculated in Step 6, spike the matrix at this calculated MDL and proceed through the procedure starting with Step 4.

(b) If this is the second or later iteration of the MDL calculation, use S^2 from the current MDL calculation and S^2 from the previous MDL calculation to compute the F-ratio. The F-ratio is calculated by substituting the larger S^2 into the numerator S^2_A and the other into the denominator S^2_B. The computed F-ratio is then compared with the F-ratio found in the table which is 3.05 as follows: if $S^2_A/S^2_B<3.05$, then compute the pooled standard deviation by the following equation:

$$S_{pooled} = \left[\frac{6S_A^2 + 6S_B^2}{12}\right]^{\frac{1}{2}}$$

if $S^2_A/S^2_B>3.05$, respike at the most recent calculated MDL and process the samples through the procedure starting with Step 4. If the most recent calculated MDL does not permit qualitative identification when samples are spiked at that level, report the MDL as a concentration between the current and previous MDL which permits qualitative identification.

(c) Use the S_{pooled} as calculated in 7b to compute The final MDL according to the following equation:

MDL=2.681 (S_{pooled})

where 2.681 is equal to t(12,1−α=.99).

(d) The 95% confidence limits for MDL derived in 7c are computed according to the following equations derived from precentiles of the chi squared over degrees of freedom distribution.

LCL=0.72 MDL

UCL=1.65 MDL

where LCL and UCL are the lower and upper 95% confidence limits respectively based on 14 aliquots.

Tables of Students' t Values at the 99 Percent Confidence Level

Number of replicates	Degrees of freedom (n-1)	$t_{cn-1,.99})$
7	6	3.143
8	7	2.998
9	8	2.896
10	9	2.821
11	10	2.764
16	15	2.602
21	20	2.528
26	25	2.485
31	30	2.457
61	60	2.390
00	00	2.326

Reporting

The analytical method used must be specifically identified by number or title ald the MDL for each analyte expressed in the appropriate method reporting units. If the analytical method permits options which affect the method detection limit, these conditions must be specified with the MDL value. The sample matrix used to determine the MDL must also be identified with MDL value. Report the mean analyte level with the MDL and indicate if the MDL procedure was iterated. If a laboratory standard or a sample that contained a known amount analyte was used for this determination, also report the mean recovery.

If the level of analyte in the sample was below the determined MDL or exceeds 10 times the MDL of the analyte in reagent water, do not report a value for the MDL.

[49 FR 43430, Oct. 26, 1984; 50 FR 694, 696, Jan. 4, 1985, as amended at 51 FR 23703, June 30, 1986]

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APPENDIX E

Sampling Methods

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Nutrients Sampling Method (TOTALS)

************************************ **(Total N&P)** ************************************

You do not need to wear sample gloves, but please do not touch the inside of the caps or bottles or the lips of the bottles with your hands.

--You **will need** the following--

- 250 ml HDPE Nutrient-Clean (Acid-Clean) Bottle
- YSI (multiparameter meter)
- GPS
- Sample Log Sheet
- Sample Tape
- Sharpie
- Peristaltic Pump (optional)
- Nutrient-Clean (Acid-Clean) Peristaltic Pump Hose (MUST have if using peristaltic pump)

--You **must collect** the following --

- Sampling Time
- ALL YSI Parameters
- Sample Depth
- Latitude/Longitude
- Person(s) Collecting Sample

---General Instructions---

Bottles and caps need to be **thoroughly** rinsed with sample water (rinse each bottle and cap **three times** with sample water). The bottles are then **filled, sealed, and labeled.** If you are using the peristaltic pump, **use Nutrient-Clean (Acid-Clean) sample hose ONLY.** Keep sample **chilled** and in a **dark place** while in the field.

---Sampling Procedure---

1. Thoroughly rinse the sample bottle **three times** with sample water
2. **Fill** the sample bottle with sample water
3. **Label** the sample
4. **Chill** the **sample** in a dark place (cooler with blue ice) until returning to the lab/condo
5. At the lab/condo Either (1) **Freeze the sample** for long-term storage OR (2) **Chill the sample** for short-term storage and immediate sample analysis

---Additional Notes---

✓ It is best to store the chilled samples in a dark place. If you are storing the sample in a refrigerator that will be opened frequently, like one at a condo, it is be best to put the samples in a box to keep light away from them so that the microbes in the sample are as inactive as possible and will be less likely to eat the nutrients in your sample.

Nutrients Sampling Method (SPECIFIC NUTRIENTS)

*********************** (PO_4^{3-}, $Si(OH)_4$, NO_3^-, NO_2^-, NH_3) ***********************

You **NEED** to wear gloves.

---You **will need** the following---

- 60 ml HDPE Nutrient-Clean (Acid-Clean) Bottle
- Nutrient-clean (Acid-Clean) Syringe
- De-ionized Water
- 0.45 μm GF/C Filter
- YSI (multiparameter meter)
- GPS
- Sample Log Sheet
- Sample Tape
- Sharpie
- Nitrile Gloves
- Peristaltic Pump (optional)
- Nutrient-Clean (Acid-Clean) Peristaltic Pump Hose (MUST have if using peristaltic pump)

--You **must collect** the following--

- Sampling Time
- ALL YSI Parameters
- Sample Depth
- Latitude/Longitude
- Person(s) Collecting Sample

--General Instructions--

The bottles and caps need to be **thoroughly** rinsed with **filtered** sample water (rinse each bottle and cap **three times** with **filtered** sample water). The bottles are then **filled, sealed, and labeled.** If you are using the peristaltic pump to collect the sample into the syringe, **please use Nutrient-Clean (Acid-Clean) sample hose ONLY. Keep sample chilled** while in the field.

--Sampling Procedure--

1. Put **gloves on**
2. **Rinse** a **nutrient-clean (acid-clean) syringe three times** with sample water
3. **Fill** this syringe **with sample water**
4. **Rinse a second nutrient-clean (acid-clean) syringe** with de-ionized water **three times [THIS STEP IS ONLY NECESSARY FOR FIRST USE OF EACH FILTER – see additional notes below]**
5. **Fill** this syringe **with de-ionized water**
6. Attach a 0.45μm GF/C filter to the de-ionized water rinsed syringe and **squirt at least 10 ml of de-ionized water** through the filter (to get rid of any nutrient signature from the filter)
7. Transfer the de-ionized rinsed filter to the first, sample-rinsed syringe filled with sample water and **filter ~15 ml of sample water** into the 60 ml bottle. Put the lid on the bottle and **shake the sample bottle**. **Decant (pour out) the water** and **repeat two** more times to rinse the bottle with **filtered** sample water
8. **Squirt EXACTLY 55 ml of sample** water into the sample-rinsed 60 ml HDPE bottle
9. **Label** the sample **(SEE LABELING CONVENTION BELOW)**
10. Either (1) **Freeze the 60 ml sample** for long-term storage OR (2) **Chill the sample** for short-term storage and immediate analysis

We use the 60 ml bottles for two different types of samples. To avoid confusion, please use: Sample Name and NUTS

Example: Our Spring NUTS

--Additional Notes--

- ✓ The filter can be used for the next sample if you purge it with at least 15 ml of new sample water
- ✓ Store the used filter in the filter packaging between sample use to prevent contamination from your working space
- ✓ The syringe can also be used for the next sample if you thoroughly rinse it with the new sample three times
- ✓ It is best to store the chilled samples in a dark place. If you are storing the sample in a refrigerator that will be opened frequently, like one at a condo, it is be best to put the samples in a box to keep light away from them so that the microbes in the sample are as inactive as possible and will be less likely to eat the nutrients in your sample.

$\delta^{15}N$ & $\delta^{18}O$ (nitrate) Sampling Method

You do not need to wear gloves, but please do not touch the inside of the caps or bottles or the lips of the bottles with your hands.

---You **will need** the following---

- 60 ml HDPE Acid-Cleaned Bottle
- YSI (multiparameter meter)
- GPS
- Sample Log Sheet
- Sample Tape
- Sharpie
- Peristaltic Pump (optional)
- Nutrient-Clean (Acid-Clean) Peristaltic Pump Hose (MUST have if using peristaltic pump)

---You **must collect** the following ---

- Sampling Time
- ALL YSI Parameters
- Sample Depth
- Latitude/Longitude
- Person(s) Collecting Sample

---General Instructions---

The bottles and caps need to be **thoroughly** rinsed with sample water (rinse each bottle and cap **three times** with sample water). The bottles are then **filled, sealed, labeled, chilled,** and then **frozen**.

---Sampling Procedure---

1. Thoroughly rinse the sample bottle **three times** with sample water
2. Fill the sample bottle with approximately 55 ml of sample water (leave head space for water expansion during freezing)
3. **Label** the sample
4. **Chill** the sample while in the field
5. **Freeze** the sample **immediately** upon returning from the field

We use the 60 ml bottles for two different types of samples. To avoid confusion, please use the following labeling convention: Sample Name and $\delta^{15}N(NO_3^-)$

Example: Our Spring $\delta^{15}N(NO_3^-)$

---Additional Notes---

✓ Nitrite will be removed from the sample in the lab using established laboratory procedures - please see: Granger and Sigman (2009) Removal of nitrite with sulfamic acid for nitrate N and O isotope analysis with the denitrifier method; Rapid Communications in Mass Spectrometry, v 23, 3753-3762.

<u>$\delta^{18}O$ & δD (water) Sampling Method</u>

You do NOT need to wear gloves, but please do not touch the inside of the vials and keep contact with the septa at a minimum.

--You **will need** the following--

- 20 ml Glass Vial
- Acid-Cleaned Septa
- Aluminum Seal
- E-Z Crimper
- 2 Liter Beaker
- Peristaltic Pump
- Peristaltic Pump Hose
- YSI (multiparameter meter)
- GPS
- Sample Log Sheet
- Sample Tape
- Sharpie

---You **must collect** the following ---

- Sampling Time
- ALL YSI Parameters
- Sample Depth
- Latitude/Longitude
- Person(s) Collecting Sample

---General Instructions---

Bottles and septa should be **thoroughly** rinsed with sample water (rinse each bottle and septa **three times** with sample water). The bottles are then **filled** under water**, septa sealed** under water**, checked** for **bubbles, crimp sealed** above water, and **labeled.**

---Sampling Procedure---

1. **Rinse the vial and septa** with sample water three times
2. **Rinse the beaker** with sample water three times
3. Put the septa and vial **into the beaker**
4. Place the peristaltic pump tubing at the **very bottom** of the vial and **fill the vial** from the bottom up, allowing the vial to overflow with sample water (overflow water volume should be at least 60 ml)
5. Once enough water is present to **cover the vial** in the beaker and at least three volumes of water have passed through the sample bottle, **tap out** air bubbles from the septa
6. Slowly **remove the hose** from the sample vial and **seal** the vial **underwater** with the septa
7. Firmly holding the septa in place, **remove the vial+septa from the beaker** and **invert to check for bubbles**. If no bubbles are present, place the aluminum seal over the septa and vial and **crimp seal** it. If bubbles are present, pour out the water from the vial and beaker and repeat the procedure from step 3 above
8. Dry the vial, label it, and **store** it in a safe place at **room temperature**

CFC Sampling Method – Bottles

Filling procedure – FOLLOW EXACTLY

Instruction given below MUST BE followed to the letter to obtain good results with the bottle sampling method for CFCs in ground water.

Make sure you have **ABSOLUTELY NO** lubricants, oils, greases, sprays, or plastic materials on your hands when you sample (including sun-block or lotion). **Do NOT** wear gloves to sample.

--You **will need** the following--

- 125 ml Boston Round Bottles (three per site)
- Plastic Aluminum-Foil-Lined Caps
- 2 Liter Glass Beaker
- Viton MasterFlex Compatible Tubing
- Electrical Tape
- YSI (multiparameter meter)
- GPS
- Sample Log Sheet
- Sample Tape
- Sharpie
- Towel
- Bubble Wrap
- Peristaltic Pump (optional)

--You **must collect** the following --

- Exact time of capping of CFC Bottle 1
- Exact time of capping of CFC Bottle 2
- Exact time of capping of CFC Bottle 3
- ALL YSI Parameters
- Collect a $\delta^{18}O$(water) & δD (water) sample
    - NOTE Exact time of sampling
- Latitude/Longitude
- Person(s) Collecting Sample

--General Instructions--

Bottles and caps need to be **thoroughly rinsed** with the sample water (rinse each bottle and cap **three times** with sample water). **Fill** the bottles **underwater** in a **glass** beaker and **cap** them **underwater**. Collect **three bottles** per well or spring. After filling one bottle, **decant (pour out)** all water in the beaker and **start over** for the next bottle. **Fill and label** the bottles sequentially starting with bottle #1.

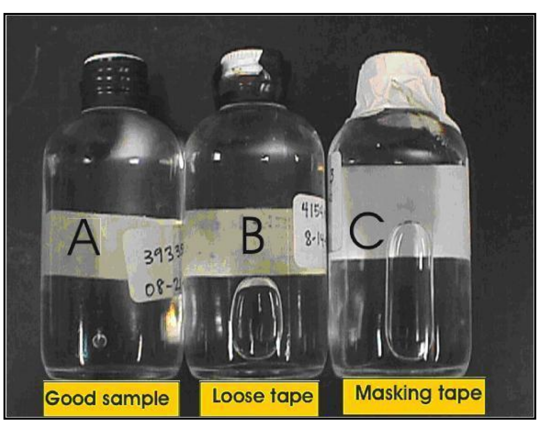
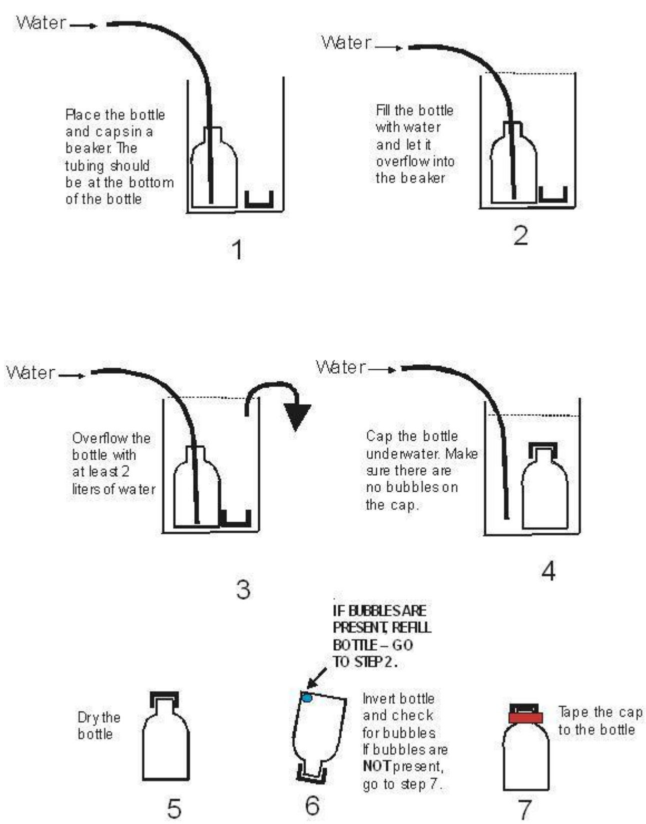


FIGURE EXPLANATION

A. Good example. Very tiny bubble formed.
B. Poorly taped cap, air leak - note the large bubble that formed.
C. Cap taped with masking tape, poor seal and large air bubble formed.

--Sampling Procedure--

1. After the well has been purged, **rinse** the sample bottle **three times** with sample water
2. **Rinse** the beaker **three times** with sample water
3. Place the bottle in the beaker and then insert one end of the **viton tubing** ALL the way to the **bottom** of the bottle
4. **Fill the bottle** until the bottle overflows (see figure below – cartoon 1)
5. Continue to **overflow the bottle until the beaker overflows**, allowing at least 2 liters of water to flow through the bottle and out of the beaker (see figure below – cartoons 2 & 3) ---- Flushing the bottle with more water is far better than with less water
6. Select a **cap and tap it under water** to dislodge air bubbles
7. **Slowly remove** the viton tubing from the bottle and **tightly cap** the bottle **underwater** without allowing the water in the bottle to come in contact with air (see figure below – cartoon 4)
8. Remove the capped bottle from the beaker, **dry the bottle** and **<u>RE-tighten</u>** the cap ---- The tighter the cap the better (see figure below – cartoon 5)
9. **Invert** the bottle, **tap it** and **check** for **air bubbles** (see figure below – cartoon 6). If there are bubbles, empty the bottle and beaker and repeat the procedure from step 3 above. If it is necessary to refill the bottle, you **MUST USE a new** cap
10. If there are no bubbles, tape the cap **securely** to the bottle with **electrical tape**. Wrap the tape in a **clockwise direction** looking down from the bottle top. **Two rounds of electrical tape** are needed (see figure below – cartoon 7)
11. **Label each bottle** with the well name, date, and time of sampling and the sequence number of each bottle as it was collected, one through three, in the order of collection
12. **Bubble-wrap each bottle** and **store them upside down at room temperature** until shipment. It is normal for a bubble to form in most samples after the sample has been stored for a short while.



<u>Radon Grab Sample Sampling Method</u>

You do NOT need to wear gloves.

--------------------You **will need** the following--------------------

- 250 ml bottle (preferably for salinity 15+)
- 40 ml bottle (use if 250 ml bottle is NOT available, but ONLY for salinity 0 to 15)
- YSI (multiparameter meter)
- GPS
- Sample Log Sheet
- Sample Tape
- Sharpie
- Filling container (optional)
- Peristaltic Pump and Hose (optional)

--------------------You **must collect** the following --------------------

- Sampling Time
- ALL YSI Parameters
- Sample Depth
- Latitude/Longitude
- Person(s) Collecting Sample

--------------------General Instructions--------------------

The bottles and caps need to be **thoroughly** rinsed with the sample water (rinse each bottle and cap **three times** with sample water). The bottles are then **filled** (typically under water)**, sealed** (typically under water)**, checked** for **bubbles**, and **labeled.** The samples **HAVE** to be **analyzed ASAP** because the half life of radon is 3.8 days.

--------------------Sampling Procedure--------------------

There are three different procedures for filling radon sample bottles. Pick the procedure that is appropriate for your sampling set-up.

IF USING PUMP/HOSE AND A FILLING CONTAINER

1. **Rinse the sample bottle and lid** with sample water **three times**
2. **Rinse the beaker/filling container** with sample water **three times**
3. Put the bottle and lid **into the beaker/filling container**
4. Run the peristaltic pump at a **very low speed** so that the Rn does not degas
5. **Put** the peristaltic pump tubing ALL the way to the **bottom** of the sample bottle and **fill** the bottle from the bottom up, allowing it to overflow with at least three volumes of sample water
6. Once at least three volumes of water have flushed the bottle and enough water is present to **cover the bottle** in the beaker/filling container, **tap out** air bubbles from the cap
7. Slowly **remove the hose** from the sample bottle, and run the hose over the cap wile under water to put fresh sample water in the cap and then **seal** the bottle **underwater**
8. R**emove** the sealed bottle from the beaker/filling container and **invert** it to check for bubbles
9. If no bubbles are present, dry the bottle, label it, and **store it** in a safe place. If bubbles are present, empty the sample bottle and beaker/filling container and repeat the procedure from step 5 above
10. **Store** the sample at room temperature
11. **Analyze** the sample ASAP

IF USING PUMP/HOSE BUT NO FILLING CONTAINER

12. **Rinse the sample bottle and lid** with sample water **three times**
13. Run the peristaltic pump at a **very low speed**

14. **Put** the peristaltic pump tubing ALL the way to the **bottom** of the sample bottle and **fill** the bottle from the bottom up, allowing it to overflow with at least three volumes of sample water
15. Slowly **remove the hose** from the sample bottle, **immediately** put sample water into the cap, and carefully flip the cap over to **seal the bottle**
16. **Invert** the bottle to check for bubbles
17. If no bubbles are present, dry the bottle, label it, and **store it** in a safe place. If bubbles are present, empty the sample bottle and repeat the procedure from step 14 above
18. **Store** the sample at room temperature
19. **Analyze** the sample ASAP

IF COLLECTING WITHOUT PUMP/HOSE AND FILLING CONTAINER (LEAST PREFERRED METHOD – LAST RESORT METHOD)

20. **Rinse the sample bottle and lid** with sample water **three times**
21. **Fill** sample bottle underwater, allowing air inside the bottle to escape and be replaced by sample water
22. **Fill** the cap with sample water and **seal** the sample underwater
23. **Invert** the bottle to check for bubbles
24. If no bubbles are present, dry the bottle, label it, and **store it** in a safe place. If bubbles are present, empty the sample bottle and repeat the procedure from step 21 above
25. **Store** the sample at room temperature
26. **Analyze** the sample ASAP

Radium Sampling Method

You do NOT need to wear gloves, but bring your muscles.

---You **will need** the following---

- Radium sample container
- Mn Fibers
- Mn Filter Cartridge
- Mn Filter Hose
- Weighing Scale
- YSI (multiparemeter meter)
- GPS
- Sample Log Sheet
- Sharpie
- Peristaltic Pump and Hose (optional)
  - Mesh Screen for the Mn Cartridge
- If Particulates are Present USE EITHER:
  - Raw (white) Mn Fibers
  - 0.45 µm GF/C filter

---You **must collect** the following ---

- Sampling Time (both start and stop)
- ALL YSI Parameters
- Sample Depth
- Latitude/Longitude
- Person(s) Collecting Sample

--General Instructions--

The bottles and caps should be **thoroughly rinsed** with the sample water (rinse each container and cap **three times** with sample water). The containers are then **filled, sealed, labeled, weighed, connected** to the Mn cartridge with Mn fibers inside, and **filtered.**

--Sampling Procedure--

The sample container filling procedure is always the same, but there are two different procedures for filtering the sample water through the Mn cartridge. Pick the procedure that is appropriate for your sampling set-up.

FILLING THE SAMPLE CONTAINER

1. **Label** the sample container with the sample name
2. **Rinse** the sample container **three times** with sample water
3. **Fill** the sample container, recording the sample filling start and stop time
4. If **particulates are present** and you are using a peristaltic pump to pump water into the sample container, you can insert a 0.45 µm filter in-line with the peristaltic pump hose to prevent the particulates from entering the sample container OR you can filter out the particulates when you filter the sample water through the Mn Cartridge (see below)
5. Put the cap on the sample container and **slowly tip** the container over to check for leaking water. If water leaks, try resealing the container with the same cap or pick a different cap and rinse it three times with sample water and try sealing the container (some caps just do not work well)
6. **Weigh** the sample container

FILTERING THE SAMPLE WITHOUT A PERISTALTIC PUMP

1. Put **Mn fibers** into the **Mn cartridge** and **label** the Mn cartridge with the sample name. If particulates are present in the sample, use a small wad of raw (white) fibers in the Mn cartridge where the water

enters the cartridge to capture the particulates (discard the wad of raw fibers once you are done with step 5 below)

2. **Connect** the Mn cartridge to the sample container
3. **Slowly tip** the sample container over to slowly fill the Mn cartridge with sample water, allowing time for the fibers to become saturated and for air bubbles in the cartridge to escape
4. Once water is near the top of the cartridge, **slowly tip the cartridge to the ground** and **adjust the flow rate** of the water so that **less than 1 L/min** of water is flowing through the cartridge (You may prefer to hang the cartridge for faster flow or let air into the sample container if the flow slows down)
5. Once all water is filtered through the cartridge, **remove the Mn fibers** from the Mn filter cartridge, **squeeze the fibers** out, and return the Mn fibers to the sample bag labeled with the sample name. **Discard** raw white fibers if you used them to filter out particulates
6. If the **sample is brackish to saline**, once the sample water has flushed through the Mn fibers, thoroughly rinse the Mn fibers with de-ionized water that has drained through a different Mn cartridge filled with Mn fibers
7. Store the Mn fibers at room temperature

FILTERING THE SAMPLE WITH A PERISTALTIC PUMP

1. Put **Mn fibers** into the **Mn cartridge** and **label** the Mn cartridge with the sample name. **Put mesh screen** at the drain end of the Mn cartridge to prevent the fibers from escaping from the cartridge. If particulates are present in the sample, use a small wad of raw (white) fibers in the Mn cartridge where the water enters the cartridge to capture the particulates (discard the wad of raw fibers once you are done with step 5 below)
2. **Connect** the Mn cartridge to the sample container
3. **Slowly tip** the sample container over to slowly fill the Mn cartridge with sample water, allowing time for the fibers to become saturated and for air bubbles in the cartridge to escape
4. Once water is near the top of the cartridge, **slowly tip the cartridge to the ground** and **adjust the flow rate** of the water so that **less than 1L/min** of water is flowing through the cartridge (You may prefer to hang the cartridge for faster flow or let air into the sample container if the flow slows down)
5. Once all water is filtered through the cartridge, **remove the Mn fibers** from the Mn filter cartridge, **squeeze the fibers** out, and return the Mn fibers to the sample bag labeled with the sample name. **Discard** raw fibers if you used them to filter out particulates
6. If the **sample is brackish to saline**, once the sample water has flushed through the Mn fibers, thoroughly rinse the Mn fibers with de-ionized water that has drained through a different Mn cartridge filled with Mn fibers
7. Store the Mn fibers at room temperature

Trace Metals Sampling Method

Please wear gloves and please do not touch the inside of the caps or bottles or the lips of the bottles with your hands.

You **will need** the following

- 100 ml HDPE Trace-Metal Clean (Acid-Clean) Bottle
- Trace-Metal Clean (Acid-clean) Syringe
- 0.45 µm GF/C filter
- YSI (multiparameter meter)
- GPS
- Sample Log Sheet
- Sample Tape
- Sharpie

You **must collect** the following

- Sampling Time
- ALL YSI Parameters
- Sample Depth
- Latitude/Longitude
- Person(s) Collecting Sample

General Instructions

If sampling around Oahu, **pre-fill** bottles with 20 µL of trace metal grade cc. HCl. Do not rinse bottles or caps with water. If shipping bottles to other locations, **do NOT pre-fill** with acid and the bottles and caps should be **thoroughly** rinsed with sample water (rinse each bottle and cap **three times** with sample water). The bottles are then **filled, sealed, and labeled.** When back in the lab, **add** 20 µL of trace–metal grade cc. HCl. **Store bottles** in plastic bags and prevent from soiling.

Sampling Procedure

1. Put **gloves on**
2. **Rinse** the trace-metal clean syringe **three times** with sample water
3. **Fill** the syringe with **sample water**
4. **Flush** the filter with one syringe volume of sample water
5. **Filter some** sample water into the sample bottle to rinse the bottle, decant (pour out) the water from the bottle and **repeat two** more times.
6. **Fill** the bottle with filtered sample water
7. **Label** the sample
8. **Put** the sample in a **plastic bag**
9. **Store** at room temperature

Additional Notes

✓ Reuse the syringe and filter for the next sample but rinse thoroughly with the new sample water

APPENDIX F

Turner Designs Model 10AU Fluorometer User's Manual

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MODEL 10-AU-005-CE FLUOROMETER USER'S MANUAL

APRIL 1999

Part Number
10-AU-074

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Section 1

INTRODUCTION

A. Getting Started

Congratulations on the purchase of your new digital fluorometer! With proper care, it should give you years of reliable service. If, at any time, you need help with your instrument, just call us. We're here to help you.

This manual was written for the 10-AU-005-CE Fluorometer. The step-by-step instructions in Section 1D will help you to get started.

B. Definition of Symbols

The symbols below can be found in this manual and notify the user of important safety information:

This terminal can receive or supply an alternating and a direct current or voltage.

This symbol indicates high voltage.

This symbol appears to indicate a "warning" to make note of important safety considerations.

These symbols indicate important user information related to proper operation of the 10-AU and not to user safety:

NOTE: $\boxed{\textbf{CAUTION}}$

C. General Precautions

Fluorescence is basically a very safe technology. However, as with any electronic equipment there are some safety issues.

1. **Electrical.** The 10-AU is powered with electrical sources ranges from 90 - 240 VAC; 50/60 Hz. High voltage in the range of 250 to 1000 VDC is present inside the unit's <u>sealed</u> electronic compartment. Perform only procedures described in this manual. To avoid risk of shock, do not attempt to open the sealed case. If the instrument is to be removed from the sealed case (recommended only for trained electronics technicians), <u>before</u> removal **UNPLUG THE UNIT AND WAIT AT LEAST 1 MINUTE.** Avoid contact with exposed electrical circuits.

2. **Ultraviolet Light.** In some applications such as Rhodamine WT and Short Wavelength Oil, the light source is supplied by a clear quartz lamp, which is a source of ultraviolet light. This light can cause permanent damage to the eyes if observed. The lamp is contained in the sample compartment, which prevents any hazard during normal operation. When the sample compartment cover is removed for any reason, a safety interlock switch turns the lamp off. **DO NOT** override this safety switch. You must wear approved protective goggles whenever there is a potential for exposure to ultraviolet light from the quartz lamp. The instrument should be turned off and unplugged before removing or changing the lamp.

3. **Chemicals.** Some applications require the use of fluorescent dyes or solvents such as acetone, or other chemical preparation. Always consult the appropriate Material Safety Data Sheets (MSDS) as supplied by the chemical supplier. DO NOT store, handle, or work with any chemicals or hazardous materials unless you have received appropriate safety training and have read and understood all related MSDS. Work in a well ventilated area, use chemicals in accordance with all federal, state, and local regulations related to chemical storage, handling, and disposal, and limit exposure to hazardous chemicals.

   When performing discrete sample measurements, use capped test tubes whenever possible to prevent the spill of any volatile or other potentially harmful chemicals. Where capped test tubes are not available, use Parafilm™ to prevent spills.

   Note that the 10-AU-005-CE's electronics are sealed so that they are protected from damage or hazard in case of a spill inside the sample compartment. In addition, the lamp compartment is separated from the sample compartment to prevent damage to the lamp components in case of a spill or leak.

CAUTION

4. The 10-AU-005-CE and its accessories are designed for use in freshwater and marine environments. When using the continuous flow cuvette system, DO NOT use organic solvents such as acetone, methanol, or pyridine, or corrosive materials such as strong acids and bases in the flow cell.

D. Getting Started--Step-by-step

This step-by-step procedure will help you carry out your FIRST study. After you have been through the procedure once, you will probably only need to do steps 8, 9, and 10 for future studies.

Before using your fluorometer, review Section 1B and 1C above for important safety information.

1. Please fill out and return the warranty sheet at the beginning of this manual. Timely return of this information will ensure that you receive prompt notice of new features, accessories, and literature.

2. Make sure you have everything you ordered by checking your shipment against the packing list. In the unlikely event something is missing, call us at once so we can help resolve the discrepancy.

   Details about your instrument's set-up (it lists things installed by Turner Designs before shipment) can be found in the Instrument Set-Up Form in the front of this manual.

3. If fluorescence is new to you, before starting you <u>may</u> want to read Appendix 2, Key Operating Principles.

4. Review Section 1E. This will tell you how to supply power to your instrument and things to consider about mounting and environment. Section 1F concerns the sample system, filters, and light source.

5. Review Section 2. This contains locations and definitions of controls and items appearing on the digital display. You might find Figure 1 (Section 2B) and the Screens Flow Chart (Section 2I) particularly useful.

   If you like, turn on the Model 10-AU and "page" through the screens as you review them in the manual.

   Section 3A discusses how the Sensitivity Adjustment Knob, ranges, and Span function work together to set the sensitivity of your instrument.

   Section 3B defines terms on the calibration screen 2.0.

6. If you wish to reset any of the operational parameters, do so at this time. See Appendix 5A for details.

7. Before calibrating your instrument for the FIRST time, you will need to set the basic operating level of your instrument using the Sensitivity Adjustment Knob. See Appendix 6B for step-by-step instructions.

8. Consider your data logging options. See Appendix 11, sections A, B, and C, for external data logging options. See Appendix 11, sections D and E, for internal data logging.

9. Calibrate your instrument. See Sections 3F.

10. You are ready to run samples. See Section 3G for routine operation.

E. Environment and Operating Conditions

1. <u>Power Requirements</u>

The 10-AU-005-CE will operate on standard 115 VAC, 230 VAC (+/-10% of the nominal voltage), 50/60 Hz, or DC power. The power cable you specified is included with the accessories.

To supply power, simply screw the appropriate power cable onto the power/telemetry connector at the front of the instrument (see Figure 1, Section 2), and attach it to the desired power source.

If using DC power and a battery, please note that the DC Power Cable with alligator clips is for <u>portable and temporary connection only</u>; it is **NOT** for permanent connection or marine use. For permanent connection or marine use, use the Marine Battery Cable only.

2. <u>Environmental Considerations</u>

Temperature. Storage temperature is -20°C to +60°C. The minimum operating temperature is 5°C; the maximum is 40°C ambient.

NOTE: **Water and Dirt.** Your fluorometer will arrive in a laboratory or a field case. Both cases are sealed to prevent water from reaching the electronics. In the field case, with the continuous-flow cuvette installed, the fluorometer will withstand waves, rain, and splashing, and can be washed off with water. The unit is **not** designed for submersion.

The inside of the Sample Compartment should be kept clean and dry. (See Appendix 7 for what to do if there is a spill.)

Movement or Vibration. The instrument can be moved and will not be affected by moderate vibration.

CAUTION

Helium. If you are working with exotic breathing mixtures, or other systems using helium, keep them away from the fluorometer because they can cause damage to the photomultiplier.

Altitude Specification: 0 - 2000 m.

Transient Overvoltages: According to Installation Category II.

Pollution Degree 2 in accordance with IEC 664.

3. Mounting Considerations

Mounting Position. For maximum stability and proper cooling of the light source, the average position of the instrument should be within 20 degrees of level.

Mounting Considerations. During normal operation, only access to the front panel is required, but be sure to allow sufficient space for easy cuvette insertion, or for cleaning the flow cell. Watch overall temperature in enclosed racks; temperature should be kept low and constant.

F. Setting Up Your Fluorometer

1. Sample System

The Model 10-AU-005-CE is configured for use with the continuous-flow cuvette system. Unless otherwise requested, your fluorometer has been shipped with the 25 mm Continuous-Flow Cuvette System installed.

The 10-AU-005-CE will also operate with a cuvette holder for discrete (test tube) samples. If you wish to use the Discrete Sample Cuvette Holder, it should be installed at this time. (See Appendix 7.)

2. Filters and Light Source

The filters and light source are installed by Turner Designs for your main application (i.e., Rhodamine, chlorophyll, oil, etc.). If you change applications, you will need to change the lamp and filters. (See Appendices 8 and 9.)

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Section 2

OPERATING INFORMATION

A. Overview

(See Appendix 2 for a discussion of operating principles.)

Please refer to Section 1 for important safety information.

The Model 10-AU Fluorometer has been designed so that no internal controls will require setting during normal operation of the instrument. All operating controls and indicators are on the front panel. (Refer to Figure 1.)

Operation of the Model 10-AU is straightforward. It consists of four basic steps:

1. **Activation and initial verification of operational parameters.** Activation involves supplying power (see Section 2E). At the time of manufacture, the fluorometer was programmed with various default operational values (see Appendix 5A). The default values should be reviewed to make sure they are appropriate for your application. Once set, you should not have to reset them unless the requirements for your study change.

2. **Setting the basic operating level.** Before calibrating for the first time, you must set the basic operating level of the fluorometer using the Sensitivity Adjustment Knob (see Appendix 6B.) Thereafter, you will set sensitivity during calibration using the keypad.

3. **Calibration.** Calibration consists of setting the sensitivity of the instrument to a level appropriate to your samples and blank, i.e., adjusting the range of concentrations you can read. Where direct concentration readout is desired, a sample of known concentration must be used as a standard. (See Section 3.)

4. **Running samples.** (See Section 3G, Routine Operation.)

B. Controls and Indicators

LIGHT CAP. Used with the Discrete Sample Cuvette Holder. It prevents external light from falling on the light detector. Keep in place even when the instrument is not in operation to prevent dirt and moisture from entering the Sample Compartment.

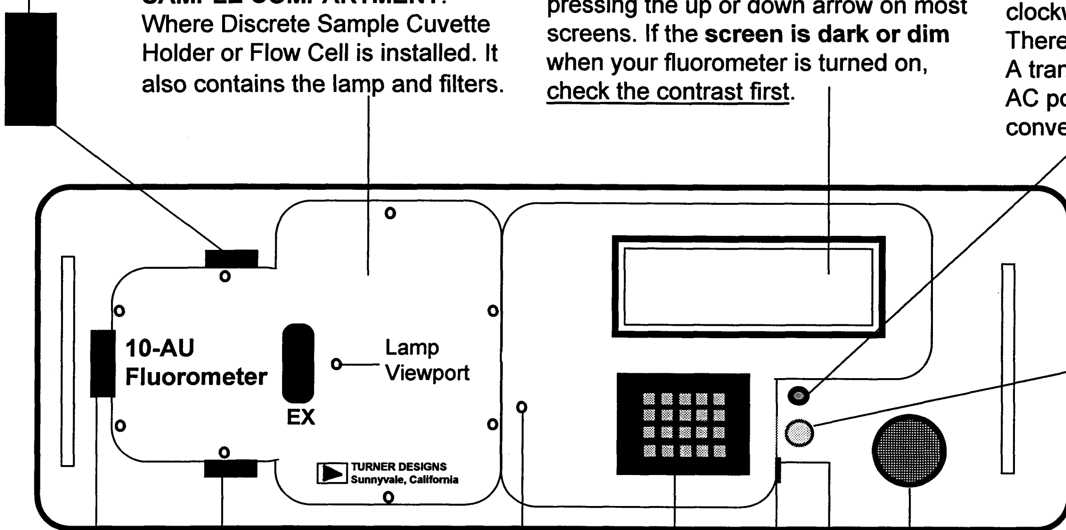
Light Cap

SAMPLE COMPARTMENT. Where Discrete Sample Cuvette Holder or Flow Cell is installed. It also contains the lamp and filters.

DIGITAL DISPLAY. The liquid crystal display (LCD) shows the screens, and displays the HOME screen. The backlight can be turned on by pressing a key on any screen. The backlight will go off automatically when a user-set time (from 10 - 3600 seconds) expires after the last keypress. *(Appendix 5A, screen 1.61.)*

Contrast of the LCD can be adjusted by pressing the up or down arrow on most screens. If the **screen is dark or dim** when your fluorometer is turned on, <u>check the contrast first</u>.

4 AMP FUSE (DC POWER). Fuse can be changed by pushing in and turning counter-clockwise. NOTE: There is no AC fuse. A transformer on the AC power cable converts AC to DC.



ON-OFF. Glows red when power is on.

EMISSION FILTER HOLDER. See Appendix 8.

SENSITIVITY KNOB LOCK. Locks the Sensitivity Adjustment Knob. MUST be locked to prevent change in basic operating level (and error in readings.)

INTAKE. For continuous flow. Drain for discrete samples.

KEYPAD. Used to enter new values and to move about among screens. The left arrow can be used as a backspace (delete) key. If the security ID is enabled, then 30 minutes after the last keypress, the instrument will return to the HOME screen. *See Appendix 5A, screen 1.64, for information about the security ID.*

POWER/TELEMETRY CONNECTOR. Contains both the power input pins and the data output pins. An AC Power and Signal Cable is standard with the 10-AU, unless another configuration is requested. To supply power, screw on the power cable and plug into power source. *(See Appendix 11 for pin inputs and outputs.)*

WRENCH HOLDER. Small opening in the casting that contains a retainer for storing the 5/32 Allen wrench (used for the Sensitivity Knob Lock and hex nuts).

SENSITIVITY ADJUSTMENT KNOB. This recessed black screw sets the basic operating level (sensitivity) of the instrument, and is locked into position by the Sensitivity Adjustment Lock. The level must be set BEFORE calibrating for the first time. Thereafter, lock it down and DO NOT adjust unless you want to change the operating level significantly, or unless you change to a different optical filter kit. After the initial adjustment of this knob, sensitivity will be set during calibration using the keypad. You MUST recalibrate your instrument if the knob is adjusted. *See Appendix 6B for instructions.*

FIGURE 1. 10-AU-005-CE Fluorometer Controls & Indicators

C. Before You Use the Software (Screens Overview)

When using the software for the Model 10-AU Fluorometer, you should familiarize yourself with a few basic instructions, which will prevent frustration and greatly speed your comprehension of the system.

1. Screens. Built into the fluorometer are a series of computerized screens, which are called up using the keypad and shown on the digital display. For easy identification, the **active screens** have an identification number in the **lower or upper right-hand corner**.

   Instructions and changes are entered on the keypad.

2. HOME Screen. Once the system has been activated, the HOME screen is continuously displayed (except when accessing other screens).

   If the security ID is enabled, then 30 minutes after the last keypress, the instrument will return automatically to the HOME screen and the security ID will have to be re-entered before any other screens can be accessed. See Appendix 5A, screen 1.64.

   If an alarm is activated when the instrument is operating, the words "ALARM ON!" will flash in the upper right hand portion of the HOME screen. Press <ESC> to access the alarm screen.

   You can **go to the HOME screen by pressing <HOME>**, except while in a help screen. **You must first exit the help screen** by pressing <ESC>.

3. Digital Display contrast. The contrast of the LCD can be adjusted on any screen, except screen 2.11 (Run Blank) and screen 2.3 (Run Standard solution), by pressing the up or down arrows.

   NOTE: If the screen is dark or dim when your fluorometer is turned on, check the contrast first. It is possible that the contrast has been decreased so much that the screen is too dark to view.

4. Main Menu. From the HOME screen, you can access the Main Menu by pressing <ENT>. From the Main Menu, the active screens may be accessed. (See the screens flow chart, following this section.)

   NOTE: If the security ID is enabled, the fluorometer will ask for an ID entry before access to screens other than HOME is allowed. See Appendix 5A, screen 1.64.

5. Moving through screens. The screens provide instructions on how to move back and forth between them; in most cases you use <ENT>. Pressing the number for a menu item accesses the screen for that function.

   You can escape to the previous screen by pressing <ESC>.

6. Left arrow. The **left arrow** can be used to **correct typing** errors when data are being entered or changed. It acts as a backspace or delete key.

7. HELP screens. The HOME screen, the Main Menu, and the calibration screens have help screens, called up by pressing <?>, which list the commands and instructions for the screens.

   NOTE: You MUST exit the help screen by pressing <ESC>, before keying other commands. The system will ignore commands given unless the help screen is exited first.

8. Warning screens. There are warning screens throughout, which will alert you to invalid entries.

9. Alarm screen. An alarm screen indicates what alarm(s) has been triggered and diagnostic screens provide information on various internal fluorometer functions. (See the Alarms & Troubleshooting section.)

10. Response delay. Under certain conditions (i.e., when sensitivity is adjusted, or instrument settings are changed, etc.), the **digital display will not react immediately** to the change, but will respond after a delay of about 10 seconds.

The software has been thoroughly tested so it is unlikely, though not impossible, that what appears to be a software malfunction is actually an inconsistency being entered into the system.

For an overview of the screens, see the screens flow chart at the end of this Section.

D. System Activation

The Model 10-AU Fluorometer is activated by supplying power and turning the instrument on. The "Turner Designs" screen will appear. The HOME screen will appear after 10 seconds, or you can press <ENT> to bring up the HOME screen immediately.

NOTE: After some years use, a warning screen will appear concerning the start-up test and non-volatile data (NVRAM). This indicates that the fluorometer internal computer battery for data storage may be low. Refer to Section 4, Alarms & Troubleshooting for details.

When the system is activated, the HOME screen will be continuously displayed.

The HOME Screen

Sample reading (with user-settable units), accurate after proper calibration. The screen can display a maximum of 3 digits, rounded off from the readings on screen 3.2 (which can support 7 digits (XXXX.XXX) for each range.) A reading greater than 999 will flash ">999". *(See Appendix 5B, screen 3.2 for a discussion of the significance of digits.)*

From screen 1.22, you may select a unit designation for your readout. *(See Appendix 5A, screen 1.2.)* You may also elect to display raw fluorescence data or direct concentration (accurate after proper calibration. If RAW is chosen, "(RAW)" will appear as the unit designation. *(Appendix 5A, screen 1.21).*

Concentration range (CONC) and whether you are operating in the AUTO or MANUAL mode. (Set range on screen 2.42; AUTO/MAN on screen 2.43.)

Time constant (see definition under screens 2.11 and 2.3)

At the option of the user, HOME may also display an analog bar graph (access screen 1.3 to change zero and full scale points, or set to auto-scale). *(See Appendix 5A, screen 1.3)*

```
CONC: MED (MAN)        XXX (PPB)
Time Const: 2 (SEC)

████████████████████████████████
0            499.5            999

<?> for help   4:42:05 PM 1/25/93
```

Time

Date

If an alarm is activated when the instrument is operating, the words "ALARM ON!" will flash here, and the audio alarm will beep unless you have turned off the beeper. Press <ESC> to view any active alarms. *(See Section 4, Alarms & Troubleshooting.)*

If you have purchased the optional internal data logger, the words "LOGGING DATA" will appear when data is being logged. *(See Appendix 11E.)*

Press <*>

Pressing <*> initiates the Discrete Sample Averaging sequence, which averages the readings over a user-settable period, and freezes the digital display for 10 seconds so you can note the reading. Thus, each sample can be read after the same amount of time has passed; and the averaging and freezing of the display minimizes both the inconvenience and potential error when readings fluctuate. When <*> is pressed, the words "Delay," then "Ave," and finally "DONE" will appear just above the units of measurement during the sequence. *(See Appendix 5A, screen 1.63.)*

Press <– (left arrow)

Call up the Full Scale Value Table indicating the maximum concentration (or raw fluorescence signal) that can be read on each range under the current calibration. *(See Section 3D, screen 2.3.)*

Press <ENT>+

```
MAIN MENU
1. Operational parameters:
2. Calibration:
3. Diagnostic information:
4. Clock:
5. Internal data logger:**

<?> for help
```

\+ If the security ID is enabled, you will be prompted for ID entry before you are allowed access to the MAIN MENU. *(See Appendix 5A, screen 1.64.)* It is the policy of Turner Designs to provide ID access information by phone if a user requests it.

** Appears <u>only if</u> you have purchased this option.

E. Verifying the Operational Parameters

The basic operational parameters have been set by Turner Designs with default values. It is possible that they may not need to be changed.

There are, however, several convenient options and you should review them initially to familiarize yourself with them, and to verify that they are set correctly for your study. (See Appendix 5A for review and instructions.)

F. Setting the System Clock

The system clock indicates the date and time, in hours, minutes, and seconds.

To set the system clock, from the HOME screen press <ENT> to call up the Main Menu. Press <4> on the Main Menu, and follow instructions.

NOTE: If you have purchased the optional internal data logging capability, and data logging is in process using the "One Way" strategy (see Appendix 11E, screen 5.3), to prevent error, the instrument will not allow you to reset the clock.

G. External Data Logger or Chart Recorder

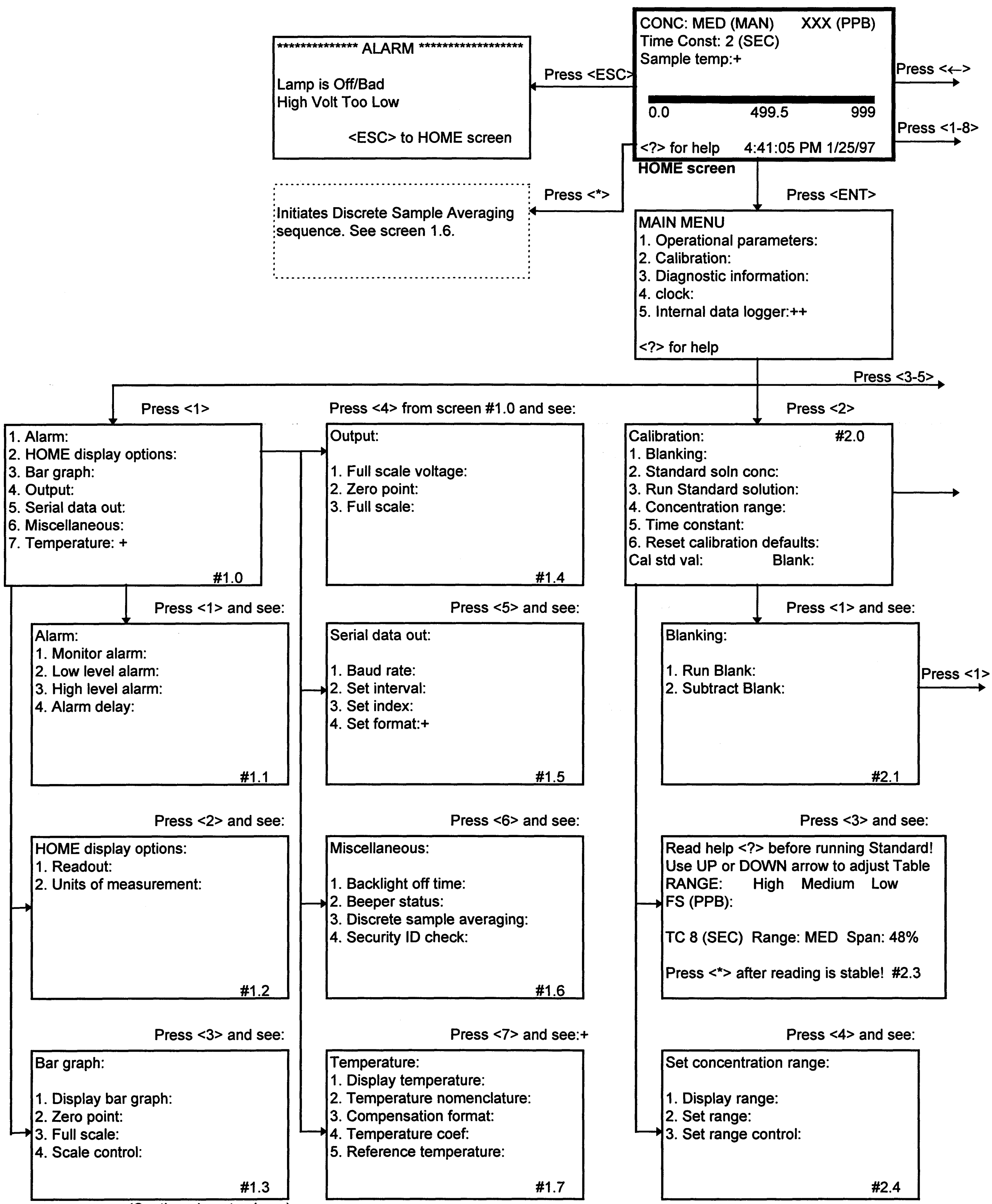
Data collection via an external data logging device or a chart recorder is an option for the Model 10-AU-005-CE. Data logging is both convenient and useful, especially in continuous-flow studies, if important data are not to be missed.

An analog signal output (user may choose a full scale output of 0.1, 1, 2 or 5 volts) is available, as is a digital output through an RS-232 serial port. There is an optional real time serial data output, which can be operated manually from the HOME screen. (See Appendix 11A, screens 1.4 and 1.5 for further information.)

H. Internal Data Logging

Internal data logging is an option on the Model 10-AU-005-CE Fluorometer. The user can log up to 64,800 data points (depending on the data logging parameters selected), including fluorescence and temperature (if the optional temperature-compensation package has been purchased). Data are retrieved through an RS-232 serial port by a PC. A software program is provided for downloading and converting the data into a Lotus-readable format.

If you have purchased this option, internal data logging parameters can be accessed through the Main Menu by pressing <5>. For an explanation of the various parameters, see Appendix 11D.



(Continued next column)

I. Screens Flow Chart

Press <←>

Full Scale Value table:
Range: Full Scale Value:
High 900.000 (PPM)
Medium 90.000 (PPM)
Low 9.000 (PPM)
Blank equivalent value:

Press <1-8>

Manual Serial Data Output

If using external data logging, sends data point with numerical marker to external computer.

+ Visible only if optional temperature compensation purchased
++ Visible only if optional internal data logging purchased
** Visible only if "One Way" internal logging strategy chosen
^^ Visible only if optional electronic chart recording purchased

From MAIN MENU

From screen 2.0, press the a number on the keypad to change the calibration factor. I.e., pressing <2> allows you to enter the standard solution concentration. Pressing <5> allows you to select the time constant.

Press <1> from screen 2.1 and see:

Use UP or DOWN arrow to set Blank% to less than 200% !
Blank %: XX

TC: 8 <SEC) Range: MED Span:48%
Press <0> after reading is stable ! #2.11

Press <3>:

Diagnostic:
Pwr level: Instr temp:
Chopper RPM: Lamp: OK
High Volt.: Within range
Ckt Status: 3609 OK
Oper hour: Oper since:
<ENT> to next screen
#3.1

Press <ENT>:

Temp. comp. readout:
Fluorecscence readout:
PM signal output:

Cal std val: Blank:
FS: XX% of XXX (PPB) at MED
Span level%:
<ENT> to next screen #3.2

Press <4> from MAIN MENU:

Set clock:
Current time and date:
6:26:43 PM 1/22/97
Set:
<1> Hour, <2> AM/PM <3> Min
<4> Month, <5> Date, <6> Year
#4.0

Press the appropriate number on the keypad to change the desired time or date factor.

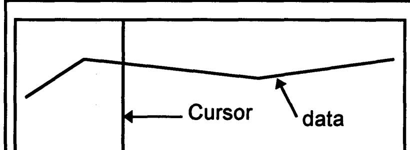
Press <5>++

Internal data logging:
1. View data:^^
2. Logging configuration:
3. Status:
Data set no. (out of 48 sets):**
Percentage of memory left:**
4. Download data to computer:
5. Erase data from memory: #5.0

Press <1> and see:^^

Strategy:
Data set no:**
Method: Interval:**
Sig. unit: (PPM) Log temp:
Starting:** (date) (time)
Ending: (date) (time)
Press <ENT> for next data set**
Press <*> to see chart #5.1

Press <*> from 5.1 and see:^^



<-<1> <2> <3>-> PgUp<4> <6>PgDn <9> 1
1/22/97 16:11:29 = 107.000 105.4 (F)

Press <2> and see:

Loggin configuration:

1. Interval:
Total data logging time:
2. Method:
3. Strategy:
4. Log temperature:+
#5.2

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Section 3

CALIBRATION AND ROUTINE OPERATION

A. Calibration Basics Using the Model 10-AU

Section 2 contains important information about the operation and features of the Model 10-AU-005-CE.

Calibration consists of compensating for blank (solution containing zero concentration of the substance to be read) and adjusting the Model 10-AU reading to reflect a known concentration of sample (the standard). During calibration you can adjust the sensitivity (Span) so you can read a desired range of concentrations.

Sensitivity of the Model 10-AU is adjusted in three ways:

a. The basic operating level of your Model 10-AU must be set using the Sensitivity Adjustment Knob before you calibrate your instrument for the first time. (See Appendix 6B.) Thereafter, DO NOT ADJUST the Sensitivity Adjustment Knob unless you change applications or cuvette size. (See Appendices 7 and 8.)

b. During calibration, the autoranging function will select the appropriate instrument range for the standard and blank. Instrument ranges (HIGH, MEDIUM, or LOW concentration ranges) are in factors of 10. As an alternative, you may set the instrument to manual range and select the range for running your standard and blank. Then, once calibrated, you may set the instrument for auto-ranging, and let it choose the best range. (See Auto-ranging, Section 3B1.)

c. During calibration, minor adjustments may be made to sensitivity on screen 2.3 using the Span function (UP and DOWN arrows).

If you are an experienced practitioner of fluorescence studies, you can proceed immediately to Section 3B.

If, however, fluorescence measurements are new to you, note that certain factors, such as temperature and the linear range of your sample, are critically important for accurate studies. When using a cuvette holder, variations from test tube to test tube can affect your readings. Please read Appendix 5A carefully before calibrating your fluorometer for the first time.

B. The Calibration Screen (Screen 2.0)

From the HOME screen, press <ENT> to call up the Main Menu. Press <2> on the keypad (Calibration) from the Main Menu. The calibration screen will appear.

```
Calibration:                          #2.0
1. Blanking:
2. Standard soln conc:      XXX (units)
3. Run Standard solution
4. Concentration range:     MED (MAN)
5. Time constant:              2 (SEC)
6. Reset calibration defaults:
Cal std val: XXX.XXX  Blank: XX.XXX
```

Actual concentration of the standard you are using. I.e., 20 ppb, 100 ppm. You enter the value on screen 2.2.

See "Run Blank," screen 2.1, next page.

See "Run Standard," screen 2.3, page 10.

The Model 10-AU is designed with three concentration ranges, HIGH, MEDIUM, and LOW, which allow measurements of samples of varying concentrations. The High range will read samples 10 times more concentrated than the Medium range, and Medium will read samples 10 times more concentrated than the Low range. The ranges are analogous to a series of maps, each more detailed (i.e., with better resolution), than the next, but covering a smaller area. The HIGH range could be thought of as a map of Europe; the MEDIUM range as a map of England; the LOW range as a street map of London. Ranges can be changed by accessing screen 2.42 and pressing <ENT>.

If set for auto-ranging, the instrument will change ranges automatically in response to varying concentrations to provide the best resolution for the sample being read. This is particularly useful for continuous-flow studies and on-line monitoring. For discrete samples, some users prefer the MAN mode.

Can be set to 1, 2, 4, or 8 seconds. One second allows you to see rapid changes in readout; 8 seconds gives more stable readings.

Calibration values can be returned to the default position on screen 2.6. Default values: FS HIGH, 900; FS MED, 90; FS LOW, 9; Span, 48%; Blank, 0.000; Cal Std Val, 50.000; standard soln conc, 15.000.

Stored value of fluorescence output for the standard as set and stored on screen 2.3, Run Standard (with blank subtracted unless you set screen 2.12 to "NO"). This value is used by the instrument to calculate direct concentration or relative fluorescence. *(See screen 3.2, Appendix 5B of the user's manual.)*

Stored value of fluorescence output for blank solution as set and stored on screen 2.11, Run Blank. This value will be used by the instrument to calculate direct concentration or raw fluorescence (unless you set screen 2.12 to "NO").

1. Auto-ranging

   Auto-ranging is a unique feature of the Model 10-AU-005-CE. If set for auto-ranging, the instrument will change ranges automatically in response to varying concentrations to provide the best resolution for the sample being read.

   If a sample reads greater than 95% of full scale on one concentration range, and remains there for at least 3 seconds with a stable signal, the instrument will automatically reduce the sensitivity of the instrument by changing to a less sensitive range: i.e., if the reading is greater than 95% of full scale on the LOW range, the Model 10-AU will automatically change to the MED range.

   If a sample reads less than 8% of full scale on one concentration range, and remains there for at least 3 seconds with a stable signal, the instrument will automatically increase the sensitivity of the instrument by changing to a more sensitive range: i.e., if the reading is less than 8% of full scale on the HIGH range, the Model 10-AU will automatically change to the MED range.

   To change your 10-AU from auto-ranging to manual or vice versa, access screen 2.43, and press <ENT> to select AUTO or MAN. When AUTO is selected, the words "(AUTO)" will appear on the HOME screen in the upper left corner next to the concentration range (unless you have turned off the range display on screen 2.41).

   When the instrument is operating in AUTO and it changes ranges, the audio alarm will beep and AUTO will alternate from AuTo to aUtO while the range is changing. The sample concentration reading will freeze at the current level for 5 seconds to avoid fluctuation in readings, and the time constant will revert to 1 second for a quicker response.

   NOTE: If the Model 10-AU changes ranges when data is being logged with an external data collection device (serial or voltage output), the "frozen" reading from the HOME screen is what will be recorded. (See Appendix 11.)

   During calibration, even though the instrument is set to AUTO, the auto-ranging function will be ***disabled*** while on the Run Blank (screen 2.11) and Run Standard (screen 2.3) screens. Note that the instrument will probably change ranges when the light cap is removed after you exit screen 2.11, and before you enter screen 2.3. For accurate readings, it is important to follow step-by-step instructions in Section 3F.

2. Calibration Screen (Screen 2.0) Defaults & Ranges

Table 1. Calibration Screen Defaults & Ranges

Value	Default	Range
Std Soln Conc.	15.000	0.00 - 999.999
Concentration Range	MED	LOW, MED, HIGH
Concentration Range Control	MAN	AUTO/MAN
Time Constant	2 (SEC)	1, 2, 4, 8 (SEC)
Cal std val	50.000	0 - 9999.999
Blank	0.000	0 - 9999.999
Span	48%	0 - 99%

C. Run Blank (Screen 2.11)

BLANKING. Blank is run on screen 2.11. Access screen 2.11 by pressing <1> from screen 2.0, then <1> again. Before measuring sample, a blank should always be collected. A blank is a sample of the water or solvent solution you will work with, taken before any of the substance to be measured has been added. This fluid should be the matrix for your standard and will be used to set the instrument to read zero. *(See Section 3F for step-by-step calibration instructions.)*

SUBTRACT BLANK. From the calibration screen 2.12, you can decide whether or not you would like the instrument to subtract blank . A "NO" setting on screen 2.12 means only that instrument will not subtract blank for you (even if you Run Blank on screen 2.11). A "YES" setting on screen 2.12 means that the instrument will subtract blank as run on screen 2.11.

The maximum blanking capability is 200% of full scale (300%), i.e., about 67% of the available signal can be used for blank, leaving 33% for samples (more than sufficient given the fluorometer's sensitivity). Once blanking is accomplished on one range, it is set for all ranges. The Model 10-AU automatically compensates for changes in concentration ranges. It is not necessary to adjust the Blank% to any particular number, as long as it is less than 200%. A low percentage number is preferred. (NOTE: The instrument will NOT prevent you from exceeding 200%.)

Tells you what range the instrument is currently operating in, i.e., HIGH, MED, or LOW. (Ideally, Blanking in LOW is recommended.)

```
Use UP or DOWN arrow to set Blank %
to less than 200%!
                    Blank%:

TC: 8 (SEC)   Range: LOW  Span: 48%
Press <0> after reading is stable !  #2.11
```

TIME CONSTANT (TC). Note that during calibration, when Span is being adjusted and $\uparrow$ or $\downarrow$ is pressed, the TC will automatically cycle through the TC values, beginning with 1 second to give you the fastest response time, then moving to 2, 4, and finally 8 (most stable and accurate). During calibration, if $\uparrow$ or $\downarrow$ is not pressed, the TC remains at 8.

Equivalent to a fine adjustment of sensitivity. Span is adjusted in a continuous manner by pressing $\uparrow$ or $\downarrow$. Press <$\uparrow$> to increase Span and <$\downarrow$> to decrease it. When minimum sensitivity is reached, the words "<MIN SEN>" will appear just above the Span; "<MAX SEN>" will appear above the Span when maximum sensitivity is reached.

D. Run Standard (Screen 2.3)

During calibration, the standard is run on screen 2.3. The Span may be adjusted if desired using <↑> and <↓> until the readings on the Full Scale (FS) Value table are satisfactory for your study. (See screen 2.3, next page.) However, if the basic sensitivity has been set according to instructions in Appendix 6B, adjusting the Span is probably unnecessary.

Normally, your standard will be a known concentration of the fluorophore of interest. For single-point calibrations, and where a known concentration is needed, we recommend you choose a standard with a concentration approximately 80% of the highest concentration you are reading, but still well within the linear range for your substance. (See Appendix 6A, Linearity.)

If you wish to read concentrations above the linear range, several standards should be used so a calibration curve may be prepared. For example, if your substance is linear to 250 ppb (parts per billion), and measurable with a calibration curve to 1000 ppb, you could calibrate with a 200 ppb standard, and take readings at 500, 750, and 1000 ppb for a calibration curve.

Calibration With a Sample of Unknown Concentration. In some procedures, *in vivo* chlorophyll or certain flow measurements, you will be calibrating with an unknown concentration. For *in vivo* chlorophyll measurements, for example, in most cases you will be calibrating with an unknown sample from the body of water you are investigating. During the calibration procedure, while sample ("standard") is flowing through the instrument and being run on screen 2.3, you should take a grab sample of the water immediately after it passes through the flow cell for later extraction to determine actual chlorophyll concentration. (**Important**: Record the *in vivo* fluoroescence reading of the "standard" as it passes through the flow cell.) You will then use a ratio method to compare all other readings with the "standard." Applying the procedures set forth in Section 3F, on screen 2.2, call your "standard" 10 or 100. Blank should be run on screen 2.11, as described. When you run your standard, use the "raw data" method.

Say, for example, you called your standard "10" on screen 2.2. Later, the standard concentration is determined by extractive methods to be 5 ppb of chlorophyll. If an unknown in the field read 12 as compared to the standard you called "10", the actual concentration of the unknown would be 6 ppb (5 ppb/10 x 12).

1. Screen 2.3 Definitions

Full scale table (FS)*. The table on screen 2.3 indicates full scale (FS)--the maximum concentration or raw fluorescence data that can be read on each of the three ranges at the current Span level. Keep in mind that the numbers are full scale values, and it is not necessary or likely that the FS values will match the concentration of your standard.

The table can support 7 digits (XXXX.XXX) for each range. The HOME screen can accommodate only three (XXX; XX.X; X.XX, .XXX). Thus readings on the HOME screen will be rounded off to 3 digits. *(See Appendix 5B for reading to 7 digits on screen 3.2.)*

Once set, values can be viewed by pressing <-- (LEFT arrow) from the HOME screen. Reset table to default from screen 2.6.

BLK > FS means that blank is higher than full scale on that range. You will not be able to use that range for sample readings. This is acceptable if blank is high and you want to read high concentrations, if the FS on the higher ranges is acceptable. *(See Section 3E3 for a way of handling high blank.)*

OVER means that the signal from the standard exceeds the sensitivity level of the instrument for that range. Reduce Span or go to a higher range. *(See Section 3F.)*

>9999 means the reading exceeds the maximum number of digits allowed. *(See Section 3F.)*

Press <-- (LEFT arrow) from HOME screen to view Full Scale Value Table.

```
Read help <?> before running Standard!
Use UP or DOWN arrow to adjust Table
RANGE:      High      Medium      Low
FS (PPB):   900.000   90.000      9.000

TC: 8 (SEC)   Range: MED   Span: 48%
Press <*> after reading is stable !  #2.3
```

Tells you what range the instrument is currently operating in, i.e., HIGH, MED, or LOW.

TIME CONSTANT (TC). During calibration, when Span is being adjusted with the <UP> or <DOWN> arrows, the TC will automatically cycle through the TC values, beginning with 1 second to give you the fastest response time, then moving to 2, 4, and finally 8 (most stable and accurate). During calibration, if the <UP> or <DOWN> arrows are not pressed, the TC remains at 8.

Span is a fine adjustment of sensitivity. If desired, Span may be adjusted by pressing the <UP> or <DOWN> arrow to increase or decrease sensitivity. NOTE: FS values increase as Span is decreased, and vice versa. When minimum sensitivity is reached, the words "<MIN SEN>" will appear just above the Span; "<MAX SEN>" will appear above the Span when maximum sensitivity is reached. If you exceed 200% for blank as currently calibrated, a warning will appear: "BLANK is more than 200%. REDUCE span!" *The instrument will not prevent you from exceeding 200% for blank.*

***Screen 2.3 FS Table Defaults and Ranges**

Concentration Range	FS Default	Range
LOW	9.000	0 - 9999.999
MED	90.000	0 - 9999.999
HIGH	900.000	0 - 9999.999

If you choose to have blank subtracted (YES on screen 2.12), the FS table readings for each range will not differ by precisely a factor of ten, because, depending on the range, blank will take up a different proportion of the total dynamic range of the instrument.

2. Screen 2.3 Examples

Screen 2.3: Example "BLK>FS"

```
Read help <?> before running Standard!
Use UP or DOWN arrow to adjust Table
RANGE:    High       Medium      Low
FS (PPB): 853.512    85.351      BLK>FS

TC: 8 (SEC)   Range: MED   Span: 48%
Press <*> after reading is stable !  #2.3
```

BLK>FS means that the blank is higher than full scale on this range. You will not be able to use this range for sample readings. This is acceptable if blank is high and you want to read high concentrations, if the FS on the higher ranges is acceptable. *(See Section 3E3.)*

Screen 2.3: Example "OVER"

```
Read help <?> before running Standard!
Use UP or DOWN arrow to adjust Table
RANGE:    High       Medium      Low
FS (PPB): OVER       OVER        OVER

TC: 8 (SEC)   Range: MED   Span: 48%
Press <*> after reading is stable !  #2.3
```

OVER means that the signal from the standard exceeds the sensitivity level of the instrument for that range. If in the HIGH range, refer to Appendix 6B to reset basic operating level before recalibrating. *(See also Calibration Procedure, Section 3F.)*

Screen 2.3: Example ">9999"

```
Read help <?> before running Standard!
Use UP or DOWN arrow to adjust Table
RANGE:    High       Medium      Low
FS (PPB): >9999     1100.000     110.000

TC: 8 (SEC)   Range: MED   Span: 48%
Press <*> after reading is stable !  #2.3
```

>9999 means the reading exceeds the maximum number of digits allowed. *(See Calibration Procedure, Section 3F.)*

E. Calibration Preliminaries

NOTE: Before calibrating, make sure that no internal function alarms are activated by checking the HOME screen. (High or low level alarms can be ignored. See the Alarms & Troubleshooting section.)

CAUTION After turning on the power, wait a minimum of 10 minutes before calibration to allow the instrument to stabilize.

1. Temperature compensation. If you have purchased the temperature-compensation package, and are working with the continuous flow-cuvette, you will need to set the temperature-related values for your study. See the Definitions section of Appendix 5A, screen 1.7. These must be set before calibration. Note that YOU MUST RECALIBRATE whenever you change the temperature format or coefficient, or the reference temperature.

2. Running Blank and Standard.

   On the 10-AU, blank and standard are run independently of each other on screen 2.11, Run Blank, and screen 2.3, Run Standard Solution. Thus, once they are set, if you want to recalibrate using a new standard but the same blank, you only need to rerun the new standard on screen 2.3. If, however, you use a new blank, re-run both the blank and the standard.

   It is good practice to set blank first, then the standard. Blank often determines the concentration range, especially in cases of high blank, and changing the blank will affect the full scale readings for your samples.

   NOTE: If you do not want the instrument to subtract blank (a "NO" setting on screen 2.12), then you do not need to Run Blank (screen 2.11).

3. High Blank. If blank is high, you may want to consider treating the high "blank" as another sample. For example, you might calibrate using distilled water as a true blank, take a reading for the high "blank" as if it were another sample, and then subtract that value manually from all your other sample readings. This method will prevent negative readings for samples (meaning they are reading less concentrated than your "blank.")

4. Direct concentration readout. When properly calibrated, the readout on the HOME screen is the direct concentration of your sample. The 10-AU contains a microprocessor, which takes the photomultiplier signal output for the sample, standard, blank, concentration range, and absolute concentration of the standard; and performs all calculations necessary. (See screen 1.21, Appendix 5A.)

5. Raw fluorescence data readout. If you like, you can choose to view the readout for your samples as raw fluorescence data. This means that what you are interested in reading is the relative fluorescence (fluorescence proportional to concentration) of one sample compared to another, rather than actual concentration. If this option is selected, the words "RAW" will appear next to the readout on the HOME screen, without a unit designation. The table on screen 2.3, Run Standard solution, will display "FS (RAW)" at the middle left, and indicate the full scale raw fluorescence readings. As with direct concentration, you can choose whether or not to have blank subtracted.

   The calibration procedure is basically the same as for direct concentration, except that on screen 2.3, a standard is useful for adjusting the Span until the sensitivity is adequate for your study, rather than equating raw fluorescence units to a known concentration.

   To select direct or raw fluorescence readout, access screen 1.21. (See also the definitions in Appendix 5A, screen 1.2.)

F. Calibration Methods

NOTE: You must set the basic operating level of the fluorometer using the Sensitivity Adjustment Knob BEFORE CALIBRATING YOUR FLUOROMETER FOR THE FIRST TIME. (See Appendix 6B.) Thereafter, it is not necessary to adjust the Sensitivity Knob unless you change cuvette sizes or change to an application using different optical filters or a different lamp. During calibration, sensitivity can be further adjusted if desired using the Span function on the keypad.

Once calibrated, the instrument will hold the calibration within instrument specifications (i.e., less than 0.5% electronic drift per month). Unless you change your blank or standard or want to change from reading very high levels to very low levels (or vice versa), you do not have to calibrate every time you read a new batch of samples. (You will, of course, need to recalibrate if you reset the basic operating level, or change the lamp or filters.) **NOTE**: Stable solid secondary standards are available from Turner Designs, which can be used to verify instrument stability.

1. Instrument Range and Standard Concentration for Calibration

Examine Figure 2 below (numbers are for example only). Within the linear range--in this case, assume that 500 is the upper limit of the linear range--sample concentration is directly proportional (linear) to fluorescence "units". On the Model 10-AU HIGH range, you can read substances up to 10 times more concentrated than what can be read on the MED range, which in turn reads substances up to 10 times more concentrated than what can be read on the LOW range.

However, "resolution" is 10 times better on the LOW range than the MED range, and 10 times better on the MED range than the HIGH range. You could think of the ranges as three different scales. Take, for example, the HIGH scale. Assume you could weigh as much as 1000 kilograms, but in 1 kilogram units, i.e., you could weigh something as 751 or 752, but not 751.3, etc. On the MED scale, you could weigh up to 100 kilograms, but on this scale you could read in 0.1 increments. On the LOW scale, you could weigh a maximum of 10 kilograms, but in 0.01 kilogram increments.

Choosing a standard concentration and instrument range:

The instrument will select the appropriate range for running your standard. Follow the step-by-step procedure in the Calibration Instructions, p. 14.

<ins>High Concentrations</ins>. If you want better accuracy in reading high concentrations, you should choose a standard closer to the upper end of the line graph in Figure 2--a concentration approximately 80% of the upper linear range would work best.

<ins>Low Concentrations</ins>. In contrast, if you are more interested in low concentrations, a standard concentration toward the low end of the line graph would be preferred.

<ins>Broad Range of Concentrations</ins>. If you wish to be able to read both high and low concentrations, while making the most of instrument capabilities, choose a standard concentration that can be read on the MED range--i.e., 50-80% of full scale on MED.

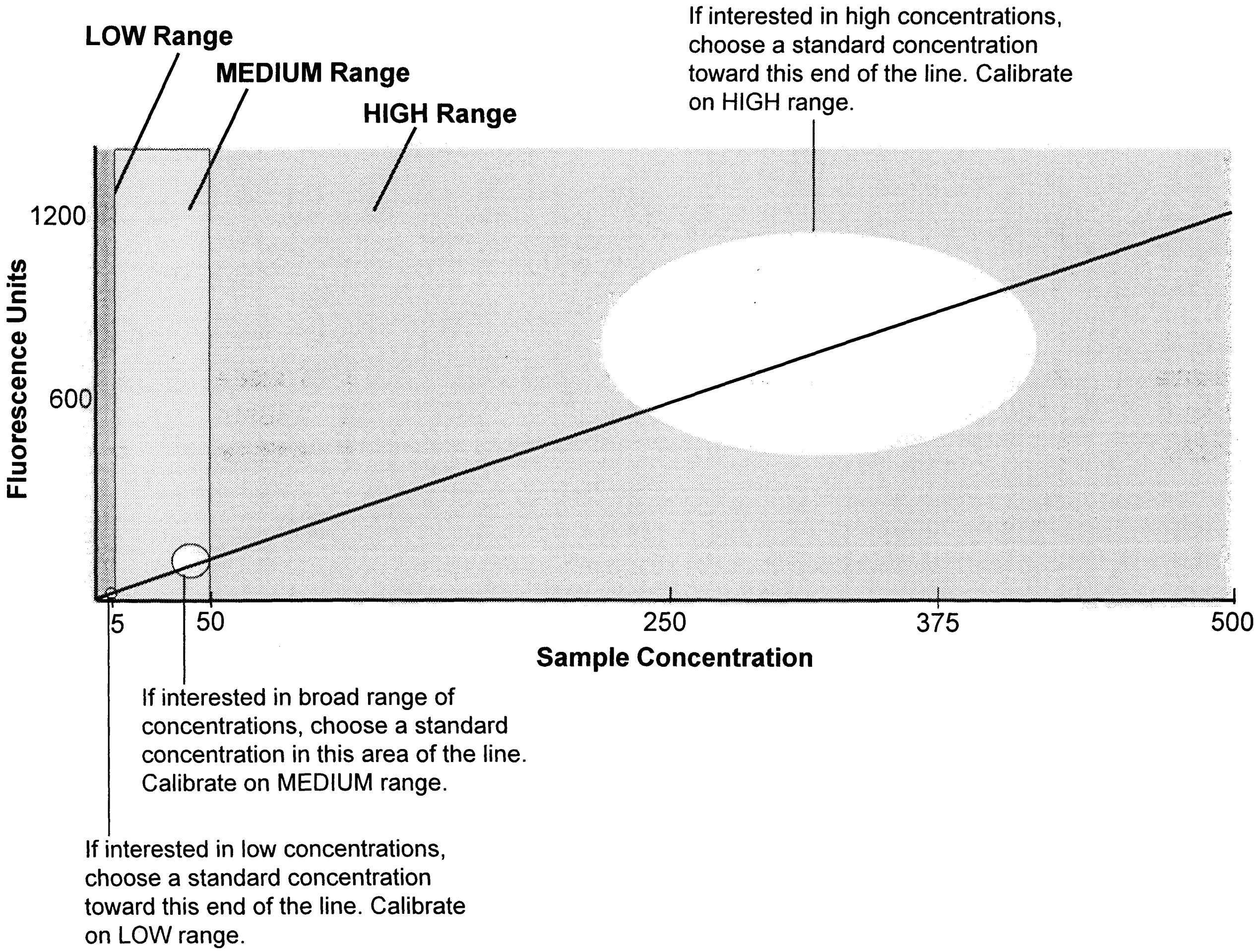

Figure 2. Instrument Range and Standard Concentration

(Sample Concentration and Fluorescence Units are for example only. Your units will vary.)

2. Recommended Standard Concentration and Instrument Range

The easiest way to calibrate is to let the instrument choose the appropriate range or ranges for running the standard and blank. As stated in the previous section, for the best sensitivity and resolution, you should prepare a standard with a low concentration (running the standard on the LOW range is preferred). If you are dealing with high concentrations, prepare a high concentration standard (running the standard on the HIGH range is preferred). If you calibration on the MED range, the instrument provides a good combination of resolution and the ability to read higher concentrations.

For example, if you want to read samples less than 3 ppb, you will get better accuracy and resolution calibrating with a 2-5 ppb standard (preferably on the LOW or MED range) than with a 75 ppb standard. However, researchers have reported good results reading low samples with the Model 10-AU Fluorometer, even after calibrating with a high standard. (The fluorometer provides good range-to-range correlation.) **Remember**: The instrument is very forgiving and flexible; in most cases good measurements will be obtained regardless of the range selected for calibration. **Note**: In all cases, for accurate readings the standard **must be** within the linear range for your substance.

It is not possible to provide an exhaustive list of recommended standard concentrations to use. However, for commonly measured substances such as chlorophyll *a* and Rhodamine WT, information about the linear range is helpful. **NOTE**: These are general and conservative guidelines only; results will vary from instrument to instrument, by optical kits, and by cuvette diameter. Linearity can be determined by a simple ratio measurement (see Appendix 6A).

	Linear Range	**Range for Calibration Curve**
Rhodamine WT[1]	100 ppb (500 ppb as 100% tracer)	500 ppb (2,500 ppb as 100%)
Chlorophyll a[2]	250 µg/L (ppb)	---

1 Rhodamine WT comes as an aqueous solution of approximately 20% active ingredient. The above concentrations assume dilutions were made considering the dye as active ingredient.

2 According to U.S.E.P.A. Method 445.0, using the Turner Designs Model 10 analog Fluorometer (13 mm tubes), chlorophyll *a* is linear to approximately 250 µg/L. (EPA research reports a higher linear range using the 10-AU.)

3. CALIBRATION INSTRUCTIONS:

Procedure	Notes & Instructions
1. Turn on the fluorometer and allow it to warm up for 10 minutes.	
2. Prepare a Standard that is approximately 80% of the highest concentration you want to read, but **still within** the linear range.	See the discussion of broad range, low, and high concentrations above; and Section 3D, screen 2.3.
3. Have on hand your Blank solution.	See the discussion in Section 3C, screen 2.11; and Section 3E3 on high blank.
4. Have on hand the samples you wish to read, with any preparation already completed.	
5. Access the Calibration menu, screen 2.0.	From the HOME screen, press <ENT> for the Main Menu, then <2> for screen 2.0.
6. Set the concentration range control to AUTO.	From screen 2.0, press <4> to bring up screen 2.4, then <3> to bring up screen 2.43 (set conc. control range), and press <ENT> to toggle.
7. Enter the <u>actual concentration</u> for your standard. I.e., 45, 100, 500, etc. (This number must be less than 1000.)	From screen 2.0, press <2> to access screen 2.2 (standard solution value). If you are reading raw fluorescence (set on screen 1.21), then set the standard solution concentration on screen 2.2 to .1, 1, or 10, with 1 preferred. The purpose of this is to allow you to read the Screen 2.3 table as a simple ratio of 10.
8. If you want the instrument to subtract Blank, make sure screen 2.12 is set to YES.	From screen 2.0, press <1> to access screen 2.1, and make sure subtract blank on screen 2.12 is set to "YES". See definition under Section 3C, screen 2.11.

Procedure	Notes & Instructions
9A. **RUN BLANK:** Determine range to run blank.	Press <HOME>. **While on the HOME screen**, insert your Blank (insert a clean, dry on the outside, cuvette containing your blank). Replace the light cap if running discrete samples. **WAIT** about 10 seconds while the instrument determines the correct range. (Lower range is preferred.)
9B. Access Run Blank screen 2.11.	Then, access screen 2.11 by pressing <ENT>, <2>, <1>, and <1>. **IF YOU WISH TO ABORT** this procedure and revert to the former calibration settings, then press <ESC> before pressing <0>. This will retain the current settings for the Span and Blank.
9C. While on 2.11, wait until the reading is stable. Then, if the Blank% (in the center of the screen) is less than 200%, **press <0>.** If not, reduce the Span by pressing the down arrow until the Blank% is less than 200%. Wait for stable reading, then **press <0> to accept the value.**	If Blank readings are very high, verify that your Blank is not contaminated. Refer to section E3 above for an alternate method for high Blank. Consider readjusting the basic operating level (Appendix 6B).
9D. Remove the cuvette and set aside.	
10A. **RUN STANDARD:** Determine range to run standard.	Press <HOME>. **While on the HOME screen**, insert your Standard (insert a clean, dry on the outside, cuvette containing your Standard). Replace the light cap if running discrete samples. **WAIT** about 10 seconds while the instrument determines the correct range.

Procedure	**Notes & Instructions**
10B. Access Run Standard Screen 2.3.	From HOME, press <ENT>, <2>, and <3>. Pressing <ESC> before pressing <*>, **WILL ABORT** the standard run and retain the current settings for the Standard.
10C. To Run Standard, wait until the readings are stable (TC cycles from 1 to 8 seconds when arrows are pressed), then **press <*> to accept the values.**	Though it is not necessary, you may adjust the Span using the up and down arrows until the FS table readings for the ranges are satisfactory. **NOTE**: Changing the Span on this screen will also affect the Blank % as set on screen 2.11; it is not necessary to re-run Blank. Remember, FS is the maximum concentration or relative fluorescence you will be able to read on a particular range, and it is not necessary or likely that FS match the "value" of the standard.
10D. Notes about Run Standard Screen 2.3	**RAW DATA.** If you are interested in raw fluorescence data, the table readings will indicate the maximum relative fluorescence you can read on each range. I.e., if you entered "1" in step 2 and the reading is 9 on the MED range, you can read a sample 9 times as concentrated as your standard. NOTE that the recommendations of when to go to a different calibration method apply here as well.
	BLK>FS. This indicates that the blank is higher than full scale at this range. This is acceptable if blank is high and you want to read high concentrations, if the FS on the higher ranges is acceptable.
	OVER. If you calibrate in manual vs. autorange, it is possible that the FS will read OVER. If the FS reads OVER, with the Span close to 0%, it means that the standard concentration is too high for the range you are in. If you are in the LOW range, change to the MED range; if you are in the MED range, change to the HIGH range. To change ranges, <ESC> from screen 2.3. Then, from screen 2.0, press <4>, <2>, and use <ENT> to toggle to the desired range. Then, run step 10.

Procedure	Notes & Instructions
10D. Notes about Run Standard Screen 2.3 (continued)	**OVER on the HIGH range.** If you are in the HIGH range and FS reads OVER, with the Span close to 0%, it means the concentration exceeds the maximum limit of detectability for the instrument. Reset the basic operating level (Appendix 6B); consider changing reference filters, or to a smaller cuvette size, or adding an attenuator plate. (See the discussion on decreasing sensitivity in Appendix 6A.)
	>9999. If FS reading exceeds 9999.999, the maximum allowable for the table, the reading will display ">9999". To get an on-scale reading, press <ESC> and reduce the standard solution concentration on screen 2.2 by a factor of 10. I.e., if it was 500, set it to 50, or 5 if necessary. If you do this, make note of the factor, as all of your samples will have to be multiplied by this factor to determine the actual concentration. Then, repeat step 10.
	Span equals 99% in the LOW range. If the Span is 99% and the reading for the LOW range is greater than 10 times the standard solution as set on screen 2.2, it probably means you will not be able to detect concentrations much less concentrated than your standard. (See discussion on increasing sensitivity in Appendix 6A.)
11. Return to HOME screen.	Press <HOME>. Run samples.

G. Routine Operation

Once the instrument is calibrated, simply insert a cuvette containing your sample into the Sample Compartment (replace the Light Cap) or start sample flowing through the flow cell, and read the concentration or raw (relative) fluorescence data on the HOME screen.

Note that the HOME screen can accommodate only three digits (XXX; XX.X; X.XX; .XXX) for sample readout. The instrument, however, can support 7 digits (XXXX.XXX) for each range. Sample readings on the HOME screen will be rounded-off to 3 digits, and a reading greater than 999 will flash ">999". Refer to Display flashes >999 in subsection 2 below if you desire more than three significant digits or are reading samples greater than 999.

The table showing the full scale available for each range as set during the last calibration can be accessed directly from the HOME screen by pressing the left arrow.

1. Auto-ranging. Setting the instrument to perform auto-ranging will give you the best possible resolution on sample readings without having to manually change ranges. (See the discussion of Auto-ranging in Section 3B1.)

   To activate auto-ranging, access screen 2.43, and set to AUTO. Refer to "Manual Operation," following, for a discussion of the meaning of "OVER," >999, or a minus (-) sign before the readout.

2. Manual Operation. If you choose to operate in the manual mode, you will have to change ranges yourself when concentrations are too high or too low for the range currently active. If you want the best possible resolution for your reading, especially on low concentrations, read your sample in the lowest range in which you can obtain an on-scale reading.

   OVER. In the manual mode, if the readout flashes "OVER," it means the concentration reads higher than full scale for the current range. If, for example, the Model 10-AU is in MED and the reading is "OVER," access screen 2.42 and change to the HIGH range. Return to the HOME screen to view the readout.

   If the reading is OVER on the HIGH range, then the concentration exceeds the upper limits of detectability of the Model 10-AU as currently calibrated. You might try diluting the sample 1:1 until you obtain an on-scale reading to get some idea of the concentration. Or, you can reduce the sensitivity of the instrument by recalibrating and reducing the Span level. Or, consider changing to a smaller cuvette size or adding an attenuator plate. (See the discussion on decreasing sensitivity in Appendix 6A.)

Display flashes >999. If the reading exceeds 999, the maximum allowable for the HOME screen, the display will flash ">999". Check the calibration table by pressing the left arrow. If the full scale reading for the current range is less than 9999.999, you can try reading your samples on screen 3.2. (See Appendix 5B, screen 3.2; and see item 4, Data Logging, below.) Or, access screen 2.2 and reduce the standard solution concentration by a factor of 10. I.e., if it was 500, set it to 50, or 5, if necessary to make the full scale reading less than 999. If you do this, make note of the factor, as all of your samples will have to be multiplied by this factor to determine the actual concentration or relative fluorescence.

Minus. If the readout on the HOME screen has a minus sign in front of it, it means that the sample is less concentrated than blank. This is more likely where you have calibrated with high blank. (See Section 3E3 for a suggested procedure for dealing with high blank.)

In cases of high blank, where the calibration table full scale value for a range reads "BLK > FS," which means than blank is higher than full scale on that range, you will not be able to use that range for sample readings.

3. Discrete Sample Averaging. Discrete sample averaging is a very useful feature for ensuring consistent readings on discrete samples, especially with temperature-sensitive samples. It allows you to average the readings over a user-settable period, and freeze the digital display for 10 seconds so you can note the reading. Thus, each sample can be read after the same amount of time has passed; and the averaging and freezing of the display minimizes both the inconvenience and potential error when readings fluctuate. See Appendix 5A, screen 1.63, to set the parameters for discrete sample averaging.

4. Data Logging. To configure the Model 10-AU for data logging using external data collection devices or the optional internal data logger, refer to Appendix 11.

   Note: You can view and save sample readings to 7 significant digits by exporting the serial data output to a computer.

5. Sensitivity Setting Retrieval. For long term studies, or where you would like to repeat a study with precision, it can be useful to be able to return to a previous instrument sensitivity setting. The diagnostic screens of the Model 10-AU make it possible to return to a former sensitivity setting simply by matching three previously noted readings.

   NOTE: In order to retrieve a setting, you must make a note of three parameters before your study is complete.

   Refer to Appendix 6C for step-by-step instructions.

6. Using Raw Data or a Ratio Method. If you are working with raw data and have calibrated with a standard of unknown concentration, you will need to perform ratio calculations to determine the actual concentration of your samples.

   Say, for example, you are measuring chlorophyll *in vivo* via continuous flow on board ship. During calibration you called your standard "10" on screen 2.2. Later, the standard concentration is determined by extractive method to be 5 ppb of chlorophyll. If an unknown in the field read 12 as compared to the standard you called "10", the actual concentration of the unknown would be 6 ppb (5 ppb/10 x 12).

Section 4

ALARMS AND TROUBLESHOOTING

A. Alarms

1. Introduction

A variety of alarms have been built into the system to warn about conditions relating to internal instrument functions or low or high sample levels.

There are two basic types: alarms that monitor internal fluorometer functions and warn of possible problems with the instrument itself; and alarms that monitor fluorescence levels and warn of levels outside of user-settable limits. To avoid unnecessary triggering of alarms, the condition must be in effect for a certain delay period.

2. Internal Function Alarms

Table 2 shows the internal function alarms, which are set by Turner Designs and cannot be changed. The status of these parameters can be checked by accessing the diagnostic information in screen 3.1. Refer to Appendix 5B for definitions.

Table 2. Internal Function Alarms

Alarm	Delay	Range
Lamp Off/Bad	1 min.	ON, Off/Bad
Chopper Speed Too Slow	2 min.	<500 rpm
Fluorometer Too Hot	5 min.	>90°C
Fluorometer Too Cold	5 min.	<-20°C
Circuit Failure	4 min.	OK, Failure
Low Power Level	1 min.	<10 % (DC: 100% = 12V; 0% = 10.2V)
High Voltage Too High	3 min.	>1000 V
High Voltage Too Low	3 min.	<25 V

3. System Alarms

Table 3 shows the user-settable system alarms. By accessing screen 1.1, the user can choose to have an alarm triggered if the sample either reaches a certain low reading (low level alarm), or a certain high reading (high level alarm). The user can also select the delay time for the alarm. Refer to Appendix 5A, screen 1.1.

Table 3. System Alarms

Alarm	Delay	Range	Default
Low Level	10-3600 sec.	0.0 - 9998.0	0.0
High Level	10-3600 sec.	1.0 - 9999.9	9999.9

4. Alarm Basics

Alarm triggered. When an alarm is first triggered, or when the condition is first corrected, the fluorometer will beep once from any screen.

When an alarm is activated and when viewing the HOME screen, the fluorometer will beep continuously and "ALARM ON!" will flash in the upper right hand portion of the HOME screen. The beeper will sound twice as fast if an internal fluorometer alarm vs. a system alarm is triggered.

Press <ESC> to view the alarm screen and take the appropriate action to clear the condition (see Troubleshooting, below).

NOTE: The beeper will not sound if you have deactivated it (from screen 1.62).

Clearing an alarm. Press <ESC> or <HOME> to return to the HOME screen from the alarm screen.

When the condition triggering the alarm is cured, the "ALARM ON!" warning will disappear from the HOME screen, and the beeper will stop.

All alarms will be reset automatically if the alarm condition is corrected. To avoid inaccurate readings, alarms cannot be stopped EXCEPT by curing the problem.

Multiple alarms. If multiple alarms are triggered, the alarm screen will list all the alarms in the order of their occurrence. Keep in mind that the triggering of certain alarms will trigger other alarms, even though there may be nothing wrong. For example:

a. If the lamp is bad, this condition will trigger the high voltage alarm (after the delay period expires), even if there is nothing wrong with the high voltage other than its relationship to the bad lamp. It will also trigger the circuit failure alarm.

b. If the chopper wheel is bad, it may also trigger either the high voltage too high or high voltage too low alarms, depending on what position the wheel is in when it stops.

c. The circuit failure alarm indicates a possible problem with the photomultiplier tube or the pre-amplifier. Depending on the problem, this may trigger either the high voltage too high or high voltage too low alarms.

Therefore, where there are multiple alarms it is IMPORTANT to deal with them in the order in which they appear on the alarm screen.

A typical alarm screen, with one alarm condition looks like this:

```
************ALARM************

Lamp is Off/Bad

                              <ESC> to HOME screen
```

An alarm screen with multiple alarms might look as follows:

```
************ALARM************

Lamp is Off/Bad
Chop. Spd Too Slow
High Volt Too High
Fluor. Too Cold
                              <ESC> to HOME screen
```

For information on what to do to correct the condition when an alarm is triggered, refer to the specific alarm condition in the Troubleshooting section, below.

B. Troubleshooting

Troubleshooting will probably begin when an alarm is triggered. The table below sets forth what to do when alarms are triggered or various problems occur. In addition to the alarms, there are two diagnostic screens that provide information on various instrument parameters. These screens can aid in identifying the source of a possible malfunction. (App. 5B, screens 3.1 and 3.2.)

To aid in troubleshooting, before contacting the manufacturer, we recommend that you fill out the diagnostic form at the end of this chapter.

Symptom	Correction
Lamp Off/Bad Alarm	Check or change lamp (Appendix 8).
Chop. Spd Too Slow Alarm	Contact manufacturer. This alarm could mean a position/speed sensor problem or a bad motor.
Fluorometer Too Hot Alarm	This alarm is triggered if the internal temperature of the fluorometer rises above 90°C (based on internal sensor: accuracy +/- 5°C). Turn off the fluorometer and allow it to cool down. If the fluorometer feels cool and this alarm is active, there could be a temperature sensor problem. Contact Turner Designs.
Fluorometer Too Cold Alarm	This alarm is triggered if the internal fluorometer temperature falls below -20°C (based on internal sensor: accuracy +/- 5°C). Make sure power is on. After start-up in cold conditions, the temperature should rise above the alarm limit within the delay period. If not, it could mean a problem with internal circuitry. Contact Turner Designs.
Circuit Failure Alarm	This alarm is triggered if there is a problem with the photomultiplier tube or the pre-amplifier. It will also be triggered if the lamp fails, or if the high voltage is too high or too low, or if the chopper speed alarm is triggered. If one of these other alarms is activated, check them first. If this does not correct the problem, contact Turner Designs.
High Voltage Too High Alarm	Check that the proper filters are correctly installed, and their condition. See Appendices 8 and 9. This alarm will also be triggered by a lamp failure. It may be triggered by a circuit failure or a chopper motor failure.

Symptom	Correction
High Voltage Too Low Alarm	Check that the proper filters are correctly installed, and their condition. See Appendices 8 and 9. It may be triggered by a circuit failure or a chopper motor failure.
Low Level Alarm	This is triggered when the sample reads at or below the user-settable concentration limit. (Alarm level is compared with the sample concentration displayed on the HOME screen.) Make sure this alarm is set where you want it, or disable it. See Appendix 5A, screen 1.1. If using the continuous-flow cuvette, check the sample delivery system (lines, connections, pump) and the flow cell for leaks (see Appendix 7).
High Level Alarm	This is triggered when the sample reads at or above the user-settable concentration. (Alarm level is compared with the sample concentration displayed on the HOME screen.) Make sure this alarm is set where you want it, or disable it. See Appendix 5A, screen 1.1. If using the continuous-flow cuvette, check the sample delivery system (lines, connections, pump) and the flow cell for leaks (Appendix 7).
Low Power Alarm	This alarm is triggered if the power level drops below 10%. Check power source (battery level if operating on DC power.)
Erratic/Noisy Readings	(No alarms activated.) Check calibration (see Section 3F). Check for air bubbles in the sample (see Appendix 6A). Check the flow cell and clean if necessary (see Appendix 7).
Unstable/Drifting Readings	(No alarms activated.) Check the reference filter (see Appendix 8). Check for moisture on the outside of the cuvette. On continuous-flow, check sample delivery system (lines, connections, pump) and flow cell for leaks (see Appendix 7).
Low Readings	(No alarms activated.) Check fluorometer concentration range. (Displayed on HOME screen, or see screen 2.4, item 2). Check calibration (see Section 3F). Check filter selection and placement (see Appendices 8 & 9). Check flow cell, and clean if necessary (see Appendix 7).

Symptom	Correction
High Readings	(No alarms activated.) If using the cuvette adapter, is the Light Cap on? Check fluorometer concentration range (HOME screen, or see screen 2.4, item 2). Check calibration (see Section 3F). Check filter selection and placement (see Appendices 8 and 9). Check flow cell for leaks (see Appendix 7).
No Response	Make sure power is on. Check power source. If using DC power, check fuse on fluorometer.
Blank or dark screen	If power is on and screen is blank or dark, try adjusting the screen contrast using the <UP> or <DOWN> arrow.
NVRAM Warning Screen	Refer to complete instructions, at the end of this section. After the fluorometer has been in use for several years, a warning screen will be automatically displayed when the instrument is turned on, which informs the user of problems with the NVRAM. This screen displays: "WARNING! NEW NVRAM, <1> to set default." This message indicates that the NVRAM internal battery may be low. The instrument will still operate, but once the battery fails, every time it is turned off it will not retain instrument settings and previous calibration settings unless power is continuously supplied to the instrument. **Temporary Solution:** The problem can be mitigated simply by leaving the instrument ON at all times, until the NVRAM module can be changed. If it is turned off, instrument settings will have to be reset and the instrument recalibrated. **Note:** When the NVRAM fails, any data stored in the internal data logger **will be saved**, as it is not dependent on the NVRAM. **Note**: On rare occasions, you may see "Some NVRAM DATA are corrupted, <1> to restore default, <0> to continue." This may indicate that there is some problem with the memory chip for storage of instrument settings. Press <1> and this may temporarily allow you to obtain readings. If readings or software performance is not normal, contact the manufacturer. Replace the NVRAM.

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Replacing the NVRAM Memory Chip

BACKGROUND. Each fluorometer contains one or two memory chips with embedded batteries. One chip, the NVRAM (non-volatile data), stores various instrument parameters such as calibration values. Some fluorometers contain a second chip, the IDL Module, which stores internal data logging values (if this option was ordered when your fluorometer was purchased). Both of these batteries will be depleted after some years use. The battery has a 10-year life specification.

NVRAM. After the fluorometer has been in use for several years, a warning screen will automatically be displayed when the instrument is turned on, which informs the user of problems with the NVRAM. This screen displays: "WARNING! NEW NVRAM, <1> to set default." This message indicates that the NVRAM internal battery is low. The instrument will still operate, but once this message appears, every time the fluorometer is turned off it will lose instrument settings and previous calibration settings.

Temporary Solution: The problem can be mitigated simply by leaving the instrument ON at all times, until the NVRAM module can be changed. If it is turned off, instrument settings will have to be reset and the instrument recalibrated. It is recommended that you write down your parameter settings immediately, in case the fluorometer is inadvertently turned off.

Note: When the NVRAM fails, any data stored in the internal data logger **will be saved**, as it is not dependent on the NVRAM.

Note: On rare occasions, you may see "Some NVRAM DATA are corrupted, <1> to restore default, <0> to continue." This may indicate that there is some problem with the memory chip for storage of instrument settings. Press <1> and this may temporarily allow you to obtain readings. If readings or software performance is not normal, contact the manufacturer and we may be able to temporarily mitigate the problem. If this screen appears, however, you should replace the NVRAM. If it appears again after a new NVRAM is installed, contact the manufacturer.

IDL MODULE. If your instrument is equipped with the Internal Data Logging option, you will need to replace the IDL module when the battery for internal data logging fails. When it fails, the menu for the Internal Data Logger will disappear from the screen, i.e., item #5 "Internal Data Logger," will not appear on the MAIN MENU; or every time the instrument is repowered, the internal data logger will appear as a new (blank) data logger. WARNING: When the data logger battery fails, any data logged which has not been downloaded will be lost. Download more frequently after the instrument has been in use for several years, to avoid loss of logged data. Replace the IDL module as the 10-year life specification of the battery approaches. Contact Turner Designs for instructions.

IMPORTANT PRECAUTIONS

WARNING!! High voltage up to 1000 volts may be present inside the instrument. Use caution and avoid the area around the large orange capacitors and lamp transformer (300 V) on the Power PCB (PCB at front of the instrument, right hand side as you face the front of the unit). Stored voltage will dissipate after a few minutes.

NOTE: It is important to follow disassembly and reassembly instructions carefully to avoid damage to internal components and impairment of instrument function.

NOTES ON CHIP HANDLING:

- Do not touch the metal pins on the chip without discharging static electricity from your hands. To discharge, touch the metal instrument chassis or handle BEFORE touching the chip.
- Chip should be stored in a clean, dry place, free of electromagnetic sources.
- Installation in the wrong orientation will damage the chip and cause a malfunction.
- Bent or improperly seated pins will cause a malfunction.

NVRAM INSTALLATION

1. If the NVRAM has not failed yet and the parameters are still set, go through each screen and write down the parameters that are currently set (so resetting parameters after installation will be easier).

2. UNPLUG THE INSTRUMENT!!!

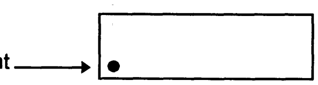
3. Remove the fluorometer from the case:
   a) Set the instrument on a clean, dry bench or table, front panel up (facing the ceiling).
   b) Remove the 18 hex-head screws on the perimeter of the front panel.
   c) Grasp the handles on the front panel of the instrument and lift it straight up. It is helpful if someone holds the case while another person lifts the unit out. **NOTE**: Make sure no wiring is caught before final removal from the case. Be careful not to damage the rubber gasket on the inside perimeter of the case.
   d) Set the instrument carefully on the bench, top side up, with the front of the unit facing toward you.

4. Face the front of the instrument. At your right, toward the back of the instrument is a corner bracket. Locate the rear-most printed circuit board (PCB). Close to the place where the corner bracket meets the rear panel there is an NVRAM in position U22 (see PCB diagram).

5. Take a small screwdriver and GENTLY pry the NVRAM from its socket. Set aside.

6. Discharge static electricity by touching the chassis. Locate the small dot on the new NVRAM. The new NVRAM <u>must be</u> installed with the dot at the lower left-hand corner as you <u>face the front</u> of the instrument.

Line up this dot so it is at the lower left-hand corner as you face the front of the instrument



7. To install the new NVRAM into the empty socket:

   a) Examine the NVRAM before installing to make sure all pins are straight.
   b) Carefully line up the bottom row of pins with the bottom row of openings.
   c) Then gently press the top row of pins into the socket.
   d) When you are sure all pins are properly seated, use the flat side of a screwdriver to press the NVRAM evenly all around, making sure it is in as far as it will go.

8. Make a visual check of the inside components to make sure no wiring is hung up and that everything appears to be secure: cable connections tight; IC's snug, etc.

9. Set the instrument case on its back and grasping the instrument handles, carefully slide the instrument back into the case. Check the rubber gasket on the case perimeter to make sure it is properly seated and undamaged.

10. Before reinstalling the 18 hex-head screws, plug in the unit and turn it on. A screen will appear announcing a new NVRAM. Press <1> and access the Main Menu and check for normal operation by paging through screens, etc. Turn the instrument off, then on again to make sure no NVRAM warning screen appears again. If a warning screen reappears, recheck your installation of the NVRAM.

11. Check the 18 hex-head screws to make sure the o-rings underneath the screw heads are present and undamaged. Reinstall the 18 hex-head screws and tighten snugly, but DO NOT overtighten and strip the nuts.

12. Reset instrument parameters and calibrate.

13. Write down the date of installation on the label supplied and affix it to the instrument.

TURNER DESIGNS
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Internet: http://www.turnerdesigns.com

Please send the following information to assist us in evaluating your instrument operation:

Client: ______________________ Contact: ______________________

Telephone: ______________________ FAX: ______________________

Instrument Serial No. ____________ RMA # ____________

Information about your standard:

Identification:______________________________

Concentration:______________________________

Lamp Part No.: ____________

Excitation filter PN: __________ Reference filter PN: ____________

Emission Filter PN: __________

Cuvette Size: 25mm 13mm 10X10 none

Type of flow cell: ______________________________

After calibrating the instrument, leave the calibration standard in the sample holder and access the diagnostic screens 3.1 and 3.2. Document the following:

Power level: __________ Instrument Temp.: __________

Chopper RPM:__________ Lamp: ______________

High Volt:______________ CKT Status: ______________

Fluorescence Readout: __________ PM Signal: __________

Cal std val: __________ Blank: ______________

FS: _____% of ________ at _____

List any alarms (press <ESC> from HOME screen to view active alarms):

Span level%:__________

Symptoms (be specific):

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Section 5

MAINTENANCE, WARRANTY, & SERVICE

A. Maintenance

To keep your Model 10-AU Fluorometer in good operating condition, the following maintenance procedures should be performed on a routine basis:

1. Clean off corrosive materials, including saltwater.
2. Check the flow cell system to make sure it is clean and that there is no evidence of leaks. (See Appendix 7.) Periodically check inside the Sample Compartment for evidence of moisture.
3. In humid areas, desiccant should be installed in the Sample Compartment area if the Continuous-flow Cuvette System is used. (See Appendix 7.) There is not much point in using desiccant with the Discrete Sample Cuvette Holder. The only way to avoid condensate with this is to have the samples at a temperature above dewpoint.
4. After some years use, the NVRAM chip for storage of calibration values and various instrument parameters will have to be replaced. See Section 4, Alarms & Troubleshooting, for details.
5. Before storing your fluorometer, remove the Sample Compartment cover and make sure the Sample Compartment is dry and free of corrosive materials (including salt). Add fresh desiccant. When you bring the fluorometer from storage, be sure to add fresh desiccant. If using a cuvette holder, tape the Light Cap securely over the opening in the top of the Sample Compartment.

B. Warranty

Turner Designs warrants the Model 10-AU series fluorometers and accessories to be free from defects in materials and workmanship under normal use and service for a period of one year from the time of initial purchase, with the following restrictions:

1. The instrument and accessories must be installed, powered, and operated in compliance with the directions in this Model 10-AU-005-CE Fluorometer User's Manual and directions accompanying the accessories.

2. Damage incurred in shipping is not covered.

3. Damage resulting from measurement of samples found to be incompatible with the materials used in the sample system is not covered.

4. Damage resulting from contact with corrosive materials or atmosphere is not covered.

5. Damage from sea water and other moderately corrosive materials that are not promptly removed from the instrument is not covered.

6. Damage caused by modification of the instrument by the customer is not covered.

7. The backlight on the digital display is warranted for 1800 hours of operation.

C. Obtaining Service

1. Warranty Service

   To obtain service during the warranty period, the owner shall take the following steps:

   a. Write or call the Turner Designs service department and describe as precisely as possible the nature of the problem.

   b. Carry out minor adjustments or tests as suggested by the Service Department.

   c. If proper performance is not obtained, ship the instrument, prepaid, to Turner Designs, with a statement of shipping charges. The instrument will be repaired and returned free of charge, along with a check to cover shipping charges to us, for all customers in the contiguous continental United States.

      For customers outside of the contiguous continental United States, and who have purchased our equipment from our distributors, contact your distributor. If you have purchased direct, contact us. We will repair at no charge, but will not pay for shipment, documentation, etc. These charges will be billed at cost.

      NOTE! Under no conditions should the instrument or accessory be returned without notice. Prior correspondence is needed:

      1. To ensure that the problem is not a simple one, easily handled in your laboratory, with savings to everyone.
      2. To specifically determine the nature of the problem, so that repair can be rapid, with particular attention paid to the defect you have noted.

2. Out-of-Warranty Service

Proceed exactly as for Warranty Service, above. If our service department can assist you by phone or correspondence, we will be glad to, at no charge.

Repair service will be billed on a basis of time and materials. A complete statement of time spent and materials used will be supplied. Shipment to Turner Designs should be prepaid. Your bill will include return shipment freight charges.

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Appendix 1
OPERATING CONDITIONS

A. Power Requirements

(See Appendix 11H for Power/Telemetry Connector pin inputs and outputs.)

~ 115 VAC. Standard power is 115 VAC, unless 230 VAC or DC is specified. The power cable has a screw-on connector for attachment to the fluorometer and 3-prong plug for connection to the AC power source.

The operating range is 100 to 130 V, 50 to 60 Hz. Virtually any generator, as well as normal household current, will supply power. The instrument is protected against transient spikes, and prolonged operation (several minutes) at 140 volts will cause no damage. AC current drain is about 0.8 amps.

The power cable is wired for use with analog and serial data collection devices. If you want to use a computer, chart recorder, or data logger, see Appendix 11A.

230 VAC is available upon request. Analogous to 115 volts, the operating range is 200 to 250 volts, 50 to 60 Hz. Current drain is about 0.4 amps.

DC Power. The 10-AU-005-CE can also be configured for 12 VDC through an optional DC power cable.

The DC operating range is 11 to 16 volts. Typical current drain is about 2.5 amperes, independent of voltage.

If using DC power and a battery, please note that the DC Power Cable with alligator clips is for portable and temporary connection only; it is **NOT** for permanent connection or marine use. For permanent connection or marine use, use the Marine Battery Cable only.

Changing Power Source. All instruments, except for certain ones manufactured for use within another system, will operate on DC as well as AC power. However, the AC power cables will not function on DC power, and vice versa.

The negative DC power line is grounded to the instrument case. Preferably, the instrument and all telemetry outputs should be isolated from the DC source. If this is not possible, ground to the negative side of the DC power source only.

B. Environmental Considerations

The 10-AU-005-CE Fluorometer is a durable field instrument designed to operate under a wide variety of environmental conditions.

Temperature. Storage temperature is -20°C to +60°C. The minimum operating temperature is 5°C; the maximum is 40°C ambient.

NOTE: **Water and Dirt.** Your fluorometer will arrive in a laboratory or a field case. Both cases are sealed to prevent water from reaching the electronics. In the field case, with the continuous-flow cuvette installed, the fluorometer will withstand waves, rain, and splashing, and can be washed off with water. The unit is not designed for submersion.

The inside of the Sample Compartment should be kept clean and dry. (See Appendix 7 for what to do if there is a spill.)

Movement or Vibration. The instrument can be moved and will not be affected by moderate vibration. The instrument is designed to operate in moving vehicles. It is important, however, to fasten it securely to prevent it from colliding with other hard objects.

CAUTION **Helium.** If you are working with exotic breathing mixtures, or other systems using helium, keep them away from the fluorometer because they can cause damage to the photomultiplier.

Altitude Specification: 0 - 2000 m.

Transient Overvoltages: According to Installation Category II.

Pollution Degree 2 in accordance with IEC 664.

C. Mounting Considerations

<u>Mounting Position</u>. For maximum stability and proper cooling of the light source, the average position of the instrument should be within 20 degrees of level.

Watch overall temperature rise in enclosed racks. Heat may be caused by other equipment. Temperatures should be kept low and constant.

<u>Access Requirements</u>. During normal operation, only access to the front panel controls is needed. Be sure to allow enough room for cleaning the flow cell or for easy cuvette insertion.

D. Sample System

Your Model 10-AU-005 Fluorometer is equipped with the 25 mm Continuous-Flow Cuvette System unless you requested another configuration.

You can change to the 13 mm or the 3 mm flow cells if you want to read higher concentrations, or to a cuvette holder for discrete sample measurements. The 13 mm & 25 mm Discrete Sample Cuvette Holder allows rapid change of cuvette sizes.

See Appendix 7 for more information about sizes available, and Appendix 10 for a discussion of the sensitivity of the various cuvettes and flow cells. Refer to the <u>Model 10-AU Digital Fluorometer Ordering Information</u> booklet for specifications.

If you wish to use one of the other cuvettes or flow cells, install it according to the instructions accompanying it, or see Appendix 7.

E. Model 10-AU-005-CE Fluorometer Specifications

<u>Alarm</u>:	User-enabled audio and visual alarm when fluorescence of sample falls below or exceeds user-settable limits. Alarm delay time: 10 - 3600 seconds
<u>Analog Output</u>:	User-settable analog output, full-scale voltage: 0.1, 1, 2, or 5 volts.
<u>Auto-Ranging</u>:	User-settable to manual or to automatic range-changing in response to changing concentration levels.
<u>Blanking</u>:	Up to 200% of signal full scale on all ranges.
<u>Continuous-Flow Cuvettes</u>:	Leak resistant cuvettes available in quartz with pathlengths of 3 mm, 10 mm, and 25 mm. Three-piece flow cells are available in pathlengths of 10 mm and 25 mm.

Digital Display:	Displays: fluorescence readout, time, date, and directions to Help screen; during internal data logging or in an alarm condition, notice is flashed. At user's option, displays: units of measurement for sample, time constant for signal averaging, concentration range, manual or auto-ranging mode, sample temperature (if equipped with temperature-compensation), and an analog bar graph.
Digital Output:	In ASCII format; through an optional RS-232 serial port. User-selectable baud rate: 4800 or 9600. User-settable, data-logging interval: 1 to 3600 seconds. Outputs are: Raw fluorescence signal units or sample concentration, date, time, index number, and temperature if fluorometer is equipped with temperature-compensation package.
Discrete Sample Cuvettes:	13 mm & 25 mm Discrete Sample Holder, which allows cuvette change in seconds. Custom adapters available upon request.
Discrete Sample Averaging:	By pressing a single key, a sample reading may be averaged over a user-settable time period for simple, accurate measurements. Pre-averaging delay: 1 - 60 seconds Averaging period: 2 - 60 seconds
Electronic Drift:	Less than 0.5% per month.
Excitation Lamp:	4-watt mercury lamp in a variety of wavelength options.

Internal Data Logging:	Purchase of internal data-logging package is optional. Instrument will log up to 64,800 data points, including index, time and date, sample readout, temperature (if equipped with temperature compensation). Data-logging interval: 1, 2, 3, 5, 10, 20, or 30 seconds; or 1, 2, 3, 5, 10, 20 or 30 minutes. Logging time: 5 hours to 1350 days (depending on data-logging parameters user sets) Electronic Chart Recording: optional with internal data logging. Allows viewing and downloading a single screen of data from the internal data logger.
Limits of detectability:	10 parts per trillion of Rhodamine WT in potable water. 10 parts per trillion chlorophyll a. 10 ppb of crude oil in water.
Operating Temperature:	Minimum: 5°C. Maximum: +40°C ambient.
Storage Temperature:	-20 to +60°C.
Physical:	Dimensions and weight vary with instrument configuration. Range:

	Minimum	Maximum
Size (in.)	21.6 x 18 x 7.7	21.8 x 19.8 x 8.75
Weight (lbs.)	24	34.5

Power, AC:	115 VAC, 230 VAC (+/-10% of the nominal voltage), 50-60 Hz.
Power, DC:	11-16 VDC; 2.5 amperes.
Ranges:	Three ranges, each a factor of 10 more sensitive than the next, covering 0 - 9999.999 fluorescence signal units.

Result Calculation:	Can provide direct concentration readout within the linear range for the substance, without the need for user-calculations; or, if preferred, raw fluorescence data.
Security ID:	User-enabled security ID to prevent unauthorized access to instrument parameters.
Self-Diagnostics:	Instrument diagnostic screens indicate: power level, chopper speed, high voltage level, circuit status, operating time, instrument internal temperature, and lamp status. Alarm is given if a malfunction occurs.
Time Constant:	User-settable to average fluorescence signal for 1, 2, 4, or 8 seconds.
Temperature Compensation:	With the optional temperature-compensation package, the instrument can correct the fluorescence output for changes in sample temperature. User may select either Celsius or Fahrenheit degrees, the temperature coefficient (linear or exponential), and the reference temperature. Temperature coefficient (linear): 0 - 15 %/°C or F Temperature coefficient (exponential): 0 - 15 /°C or F Temperature accuracy: ±0.4°F Nonlinearity: ±0.35°F (from -50 to 300°F)

Appendix 2
KEY OPERATING PRINCIPLES OF THE MODEL 10-AU

The following explanation is written for Model 10-AU users who are interested in some of the inner workings of the instrument but do not have a laboratory or instrument background. It is not intended to be a thorough course on fluorometry, but rather an explanation that will make you feel more comfortable with the instrument as you use it.

Fluorescence

The Model 10-AU Fluorometer measures the concentration of various analytes in samples of interest via fluorescence. A fluorescent molecule has the ability to absorb light at one wavelength and almost instantly emit light at a new and longer wavelength.

Light (exciting light) from a light source (the lamp) is passed through a color filter (excitation filter) that transmits light of the chosen wavelength range (color). The light passes through the sample, which emits light proportional to the concentration of the fluorescent material present and proportional to the intensity of the exciting light. (But see Linearity, in Appendix 6A.)

The emitted light goes out in a sphere. That which is headed for the detector (usually at a right angle to the exciting beam) is passed through another optical filter (emission filter). The purpose of the emission filter is to prevent any scattered exciting light from reaching the detector (in this case a photomultiplier tube) and to pass the emitted color that is specific to the analyte of interest.

The photomultiplier tube looks something like a vacuum tube, which you may have seen in communications or laboratory equipment. Like a simple phototube or photodiode, it generates electrons (electric current) in response to photons (light). What is different about a photomultiplier tube, however, is that it contains many stages (in this case, nine), each of which multiplies the electrons coming from the previous stage. Thus the current is multiplied many times before the amplifier in the fluorometer has to take over.

The wavelength of the exciting light that falls on the sample is set by the choice of the light source and the excitation filter. This wavelength is chosen (1) for strong absorption by the material under study, and (2) for minimal absorption by any interfering fluorescent materials that may be present.

The choices of photomultiplier and emission filter are made so that (1) they respond as much as possible to the light emitted by the material under study, (2) they respond as little as possible to the emission of any interfering fluorescent materials which may be present.

Refer to Figure A1 to see the optical system of the Turner Designs Model 10-AU Fluorometer.

Stability

While the process just described is straightforward, it is challenging to provide an instrument that measures sample with great sensitivity and stability under harsh conditions with less than perfect power supplies. The Model 10-AU Fluorometer achieves stability (minimal drift) by recalibrating itself 10 times a second.

When you are in the middle of a measurement and you have difficulty with your power supply or some other environmental condition, you may wonder if this affects the accuracy of your results. In most cases, it does not, because the instrument is constantly recalibrating itself. It does this by continually looking at the light that passes through the flow cell, then looking at a reference light (that comes from the <u>same</u> light source), and then at total darkness. In a sense, it triangulates itself using these three readings to stay at the same electronic reference point.

Since the same light source and detector are involved in both the measurement and reference path, variations in intensity of the lamp and in sensitivity of the detector are automatically compensated for. This is no little feat when you consider that the sensitivity of a nine-stage photomultiplier tube varies with the ninth power of the voltage.

Sensitivity

The Model 10-AU Fluorometer is highly sensitive. It can measure samples with either very low concentrations or very high concentrations of the analyte of interest, without operator recalibration. Again, the photomultiplier tube is at the heart of this process.

An initial adjustment is made to the basic operating level (sensitivity) using the Sensitivity Adjustment Knob (Appendix 6B), and the final adjustments are made on the keypad during calibration. See the Calibration section of the main text for a discussion of concentration ranges and Span adjustment. (Section 3.)

If you are interested in knowing more, consult the references below.

Why Is Fluorescence So Sensitive?

Any compound that can be measured in a fluorometer can also be measured in a colorimeter. After all, the compound has to absorb light in order to fluoresce.

Fluorescence, however, is as much as 10,000 times more sensitive.

A colorimeter (or spectrophotometer) does not measure absorbed light. It measures the transmitted light and subtracts this from the 100% (blank) transmission to get the absorbed light.

For example, you wish to measure the distance between two marks only 0.01 inch apart. The way the spectrophotometer would do it would be to measure from each of them to the wall across the room. It would then subtract these two measurements to get the desired answer. Thus, relatively small errors (on a percentage basis) would totally invalidate the answer.

The fluorometer, in effect, simply uses a micrometer caliper and directly measures the distance between the marks.

Fluorometry References

1. G. K. Turner, "Measurement of Light From Chemical or Biochemical Reactions," in Bioluminescence and Chemiluminescence: Instruments and Applications, Vol. I, K. Van Dyke, Ed. (CRC Press, Boca Raton, FL, 1985), pp. 43-78.

2. J. R. Lakowicz, Principles of Fluorescence Spectroscopy (Plenum Press, New York & London, 1983).

3. I. B. Berlman, Handbook of Fluorescence Spectra of Aromatic Molecules (Academic Press, New York & London, Second Edition, 1971).

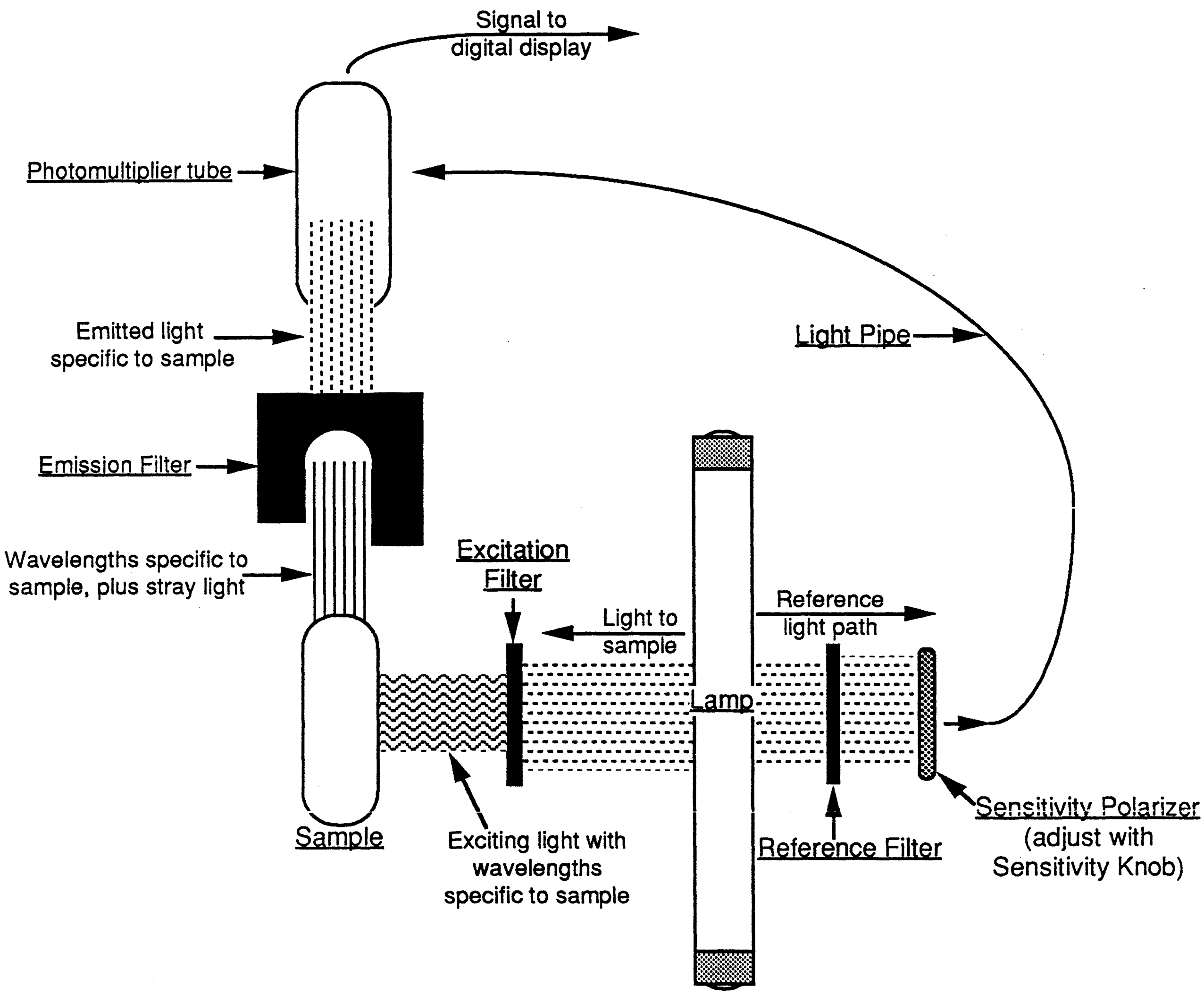

Figure A1. Optical System of the Model 10-AU

Appendix 3
STUDIES USING THE MODEL 10-AU

The Turner Designs fluorometers have been used for a wide variety of laboratory and field studies. The following are common, but by no means exhaustive, uses for the fluorometer. For information about the limits of detectability using the Model 10-AU, refer to Appendix 10, or ask us for one of our informational monographs. For your convenience, there is an order form for information at the end of this Appendix.

A. Chlorophyll and Pheophytin Studies

Fluorometric techniques have many advantages for both qualitative and quantitative measurements of chlorophyll and its primary degradation product, pheophytin. These techniques are relatively simple compared with spectrophotometry, as well as faster and more sensitive.

Lab cultures in 25 x 100 mm test tubes can be read directly in the fluorometer without the disruption of a transfer to another tube.

In many applications, the fluorometer can be used <u>in vivo</u>, with the continuous-flow cuvette, eliminating delays for extraction and processing.

The US Environmental Protection Agency has authored a new standard method for extracted chlorophyll. Contact Turner Designs for Method 445.0, "*In Vitro* Determination of Chlorophyll *a* and Pheophytin *a* in Marine and Freshwater Phytoplankton by Fluorescence."

For chlorophyll *a* measurements where chlorophyll *b* is present, another new method, "Fluorometric Analysis of Chlorophyll *a* in the presence of Chlorophyll *b* and Pheopigments," provides a reliable, effective alternative to conventional acidification techniques. (Based on research by Dr. Nicholas A. Welschmeyer of Moss Landing Marine Laboratories, Moss Landing, CA.) Acidification of extracted samples is not necessary; the procedure is sensitive enough for oligotrophic environments; and only a single fluorescence determination is required. Contact Turner Designs for details.

For more information, ask for the monograph, <u>Fluorometric Facts</u>: "Chlorophyll and Pheophytin."

B. Oil Measurements

The aromatic fractions of petroleum products are fluorescent. Without extraction, oils and gasoline, and substances such as benzene, toluene, and naphthalene can be measured conveniently with the Model 10-AU. Contact Turner Designs for more information.

Following an oil spill, the aromatic fractions dissolve in the water column and move separately from the unsightly slick on the surface. Since no sample preparation is required, the series 10 fluorometer has become very popular for tracking these fractions, allowing for protective measures for downstream water supplies and shellfish beds. It is also commonly used to map a spill following the use of dispersants. Other uses include performing baseline oil studies; pinpointing oil leaks and natural seeps; and measuring oil in organisms, sediment, and air. For more information, contact Turner Designs.

The 10-AU-005-CE with the continuous flow cuvette and TD-4100 on-line unit have been used to monitor oil in process water and in cooling water.

C. Flow Measurements and Fluorescent Tracer Studies

With the continuous-flow cuvette, the series 10 fluorometer has been used extensively with fluorescent dyes, and has proven to be an efficient and cost-effective means of flow measurement and pollution control. It can be used to calibrate flow meters on site; calibrate weirs and flumes in the field, correlate stream-level gauges with the flow rate; measure stream, canal, drainage ditch, and sewer flow directly; study sewer system infiltration; study time-of-travel in streams; and measure residence time in settling basins and disinfection chambers. For more information, ask for the monograph: "A Practical Guide to Flow Measurement."

D. Process Control

The Model 10-AU-005-CE can be used for on-line monitoring of industrial processes. It can be operated continuously over extended periods of time with minimal operator supervision.

Ask us about the TD-4100 on-line monitor for hydrocarbons, and the TD-4300 AlgaeMonitor, which are specially configured with alarms and a 4-20 mA signal output for on-line or industrial use.

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Free Information from Turner Designs

For more information

Mark the publications of interest, fill in your name and address, and FAX it to Turner Designs at 408/749-0998. Publications will be mailed to you. See the reverse side of this page for more options.

Name
Title
Company
Address
City
State, Zip
Postal Code, Country
Phone
FAX
E-Mail

Chlorophyll Monographs

____ Chlorophyll (Overview of applications) (998-5123)
____ An Instrument System for High-Speed Mapping of Chlorophyll *a* and Physico-Chemical Variables in Surface Waters (998-0033)
____ Fluorometric Analysis of Chlorophyll *a* in the Presence of Chlorophyll *b* and Pheopigments (998-0051)
____ Evaluation of a New Fluorometric Technique that uses Highly Selective Interference Filters for Measuring Chlorophyll (998-0570)
____ Chlorophyll and Pheophytin (Bulletin 101) (998-5101)
____ Algae Management in Reservoirs and Lakes (Bulletin 105) (998-5105)
____ Using the Turner Designs Model 10 Analog, The 10-AU Digital, or the TD-700 Fluorometer with EPA Method 445.0 (998-6000)
____ A Procedure for Measuring Extracted Chlorophyll *a* free from the Errors Associated with Chlorophyll *b* and Pheopigments (998-9000)

Fluorescent Tracer Monographs

____ Fluorescent Tracer Studies (Overview of applications) (998-5121)
____ A Practical Guide to Flow Measurement (998-5000)
____ Using the Turner Designs Model 10-AU Fluorometer to Perform Flow Measurements in Sanitary Sewers by Dye Dilution (998-5001)
____ Fluorometry in the Water Pollution Control Plant (998-5102)
____ Fluorescein (998-5103)
____ Fluorescent Tracer Dyes (998-5104)
____ Circulation, Dispersion and Plume Studies (998-5106)
____ Flow Measurements in Sanitary Sewers by Dye Dilution (998-5107)
____ Preparation of Standards for Dye Studies Using Rhodamine WT (998-5111)

Free Information from Turner Designs

Hydrocarbon Monographs

____ TD-4100 Technical Update No. 3 (998-4103)
____ TD-4100 Technical Update No. 2 (998-4105)
____ TD-4100 Technical Update No. 1 (998-4106)

Luminometry Monographs

____ An Evaluation of the Performance of Ten Commercial Luminometers (998-5212)
____ ATP Measurements: Theory and Practice (998-5202)
____ Applications of Beetle Luciferases as Genetic Reporters (998-5209)
____ Review: Reporter Genes; Application to the Study of Mammalian Gene Transcription (998-5215)

Application Notes

____ Application Note: A Method for DNA Analysis (998-2600)
____ Application Note: A Method for EnzChek™ (998-2601)
____ Application Note: A Suggested Method for Aflatoxin Analysis (998-2610)
____ Application Note: A Method for PicoGreen ® (998-2630)
____ Application Note: A Method for NanoOrange ™ (998-2650)
____ Application Note: A Method for Quantitation of Green-Fluorescent Protein (998-2670)
____ Application Note: A Method for CyQuant ™ (998-2672)
____ Application Note: A Method for RNA (998-2675)
____ Application Note: A Suggested Method for Histamine (998-7700)

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Appendix 4
ACCESSORIES FOR THE MODEL 10-AU FLUOROMETER

Turner Designs carries accessories for all aspects of the Model 10-AU series fluorometers. Refer to the Turner Designs Model 10-AU Digital Fluorometer Ordering Information booklet at the end of this manual.

A. Batteries for DC Power

For DC operation, the Model 10-AU Fluorometer will function properly on any portable generator providing 110-130 volts AC at 50-60 Hz, or battery that will provide 11-16 volts DC at 2.5 amperes. The negative lead should be grounded.

For portable applications, a battery is more commonly used. The prime requirement is that it must deliver 2.5 amperes for the period of expected operation without having the voltage drop below 11.

A 12-volt lead-acid battery is probably the best choice for most applications. One example is a battery designed for snowmobiles, with special caps to prevent any battery acid loss. The Gould SN-9L is rated at 32 amp/hours, weighs 21 pounds, and is 7 3/4" x 5 1/4" x 7 1/4". It has a life of about 12.8 hours.

A disadvantage of this battery and all automotive-type batteries is that they are not designed for complete discharge without damage unless they are recharged immediately after each discharge.

A battery designed especially for field work, permitting complete discharge without immediate recharge, is the Globe GC-1220B 20-ampere hour battery. It weighs 16 pounds, and is 7" x 6 1/2" x 5". It has a life of about 8 hours.

B. Pumps for Continuous-Flow Systems

Normally, sampling using the Model 10-AU 25 mm Continuous-Flow Cuvette System is done with a pump.

Flow Rate. We are frequently asked what flow rate is appropriate for continuous measurement in a flow cell. With two exceptions (discussed below), it does not matter.

Fluorescence typically occurs about ten nanoseconds after the molecule absorbs the exciting light. For a molecule to be excited on entrance to the active zone of the continuous flow attachment, and exit prior to emission, would require a flow rate of approximately 500,000 gallons per minute.

The flow rate to be used, therefore, is at the discretion of the operator. The flow rate will be a compromise between the desire to minimize transit time and the pump power and size available. The maximum flow rate that will not exceed the pressure rating of the 25 mm continuous-flow attachment (25 psig) is approximately 150 gallons per minute (570 liters/minute).

The two exceptions, where rate of flow could be important, are:

1. Where measurements are particularly temperature sensitive, as with Rhodamine and chlorophyll. In these cases, with extremely low flow the fluorometer could raise the temperature of the sample during passage through the flow cell. Although this has not been precisely tested, we expect that a minimum flow of 50 milliliters/minute is safe.

2. In the *in vivo* measurement of chlorophyll, where certain species of phytoplankton exhibit an induction effect if a long opaque hose is used (i.e., the organisms partially dark-adapt). It is recommended that the flow rate not exceed 600 milliliters/minute. High flow rates may yield falsely high readings. Refer to the section on Continuous-Flow Sampling Methods in the monograph "Chlorophyll and Pheophytin," available from Turner Designs.

Centrifugal pumps. These are the least expensive and best suited for use with the Continuous-Flow System. No matter what system is used, keep in mind that the presence of air bubbles will affect measurements; occasional bubbles are not a problem, but continuous, numerous air bubbles will invalidate the measurements.

Submersible pumps. Often used and very satisfactory. A commonly used pump for shallow sampling is a battery-operated bilge pump. Capacity is not important as long as the pump will operate against the head. Remember that once started, and returning to the surface, the head is zero. The problem is that a bilge pump will need help in getting the system primed if the fluorometer is much more than about four feet above the surface. An adequate capacity is 400 gallons per hour. Turner Designs supplies an appropriate sample pump. Refer to the Turner Designs Model 10-AU Digital Fluorometer Ordering Information booklet for specifications.

Above-water pumps. Satisfactory, although they frequently introduce bubbles by air leakage and cavitation. Therefore, it is recommended that this type of pump be mounted on the discharge side of the fluorometer. Keep in mind, also, that the sample is under suction and there is some danger of bubble formation, unless the rate of sampling and the operating head are kept relatively low.

C. Hoses

In dye studies, adsorption of Rhodamine WT is not normally a problem in nature. It is not adsorbed significantly on suspended solids. It is, however, adsorbed by soft vinyl tubing (e.g., Tygon) and by rubber and most rubber substitutes. Thus, if you use sample tubing of such material with a high concentration of dye, it may be some time before the fluorometer reading returns to a true background, even though you are sampling an area where there is no dye. In other words, it will take up some dye, then gradually bleed it out. The error will depend on the sampling rate. If the flow rate is high, the error may be minimal.

Polypropylene and high-density polyethylene do not adsorb the tracer. They are, however, stiff and somewhat difficult to work with. The most common sample hose is green garden hose. If you accidentally contaminate it with a high concentration and you must go quickly to low levels, simply be prepared to replace it.

Rubber hose is not recommended.

The hose should be completely opaque, or the portion attached to the intake and exhaust fittings of the fluorometer must be wrapped carefully with black tape. Wrapping a distance of three or four feet from the fluorometer fittings is generally satisfactory, depending on the diameter. The object is to prevent outside light from reaching the photomultiplier tube. To check, shade the hose with the instrument on a sensitive range - direct sunlight and shade should give the same reading.

On the Model 10-AU, both intake and exhaust fittings are 1/2" male pipe thread. For laboratory studies, where smaller intake tubing might be desired, Turner Designs makes a tubing adapter that will accept 3/16" to 1/4" (ID) plastic tubing. Refer to the <u>Turner Designs Model 10-AU Digital Fluorometer Ordering Information</u> booklet.

D. Dye Injection Pumps

For some studies using fluorescent dyes, a dye injection device is very useful. For flow rate measurements, an accurate injector is mandatory. There are three basic types of constant-rate injectors: constant displacement pumps, constant-head (gravity-feed) devices, and regulated pressure systems. Turner Designs carries a durable, battery-operated constant displacement injection pump. Refer to the <u>Turner Designs Model 10-AU Digital Fluorometer Ordering Information</u> booklet for specifications.

To decide on the appropriate dye injection device for your study, refer to the section on Dye Injectors in Fluorometric Facts: "A Practical Guide to Flow Measurements," available at no charge from Turner Designs.

E. Recorders, Data Loggers, and Computers.

(See Appendix 11A for information about using data collection devices with your Model 10-AU.)

The Model 10-AU Series Fluorometer will operate virtually any recorder. An integrating feature may be desirable for some studies, but special features found on some more expensive recorders, such as logarithmic presentation, X-Y recording, and scale expansion are of little value.

The recorder should be a linear strip-chart recorder with an accurate time drive. A range of chart speeds is valuable, as the speed of paper drive can be adjusted to fit the experiment. A range of 0.05 inch per minute (3 inches per hour) to 2 inches a minute (10 feet per hour) should cover most studies.

An IBM-compatible pocket data logger can also be used to record fluorometer readings.

The Model 10-AU serial data output can be used with an PC-compatible computer or printer, or a Macintosh-compatible computer with a communication program installed.

Appendix 5
OPERATIONAL PARAMETERS AND DIAGNOSTIC INFORMATION

A. Operational Parameters

To access the operational parameters, with the HOME screen displayed, press <ENT> to see the MAIN MENU, then press <1>. Press the number of the operational parameter to view it, and follow instructions on the screen to change it. For example, to access screen 1.31, after calling up screen 1.0, press the <3>, then <1>.

The operational parameters are found on the following screens. Refer to Table 4 for defaults and ranges, and to the definitions section following the table.

Screen 1.1: Alarm

- 1.11 Monitor alarm
- 1.12 Low level alarm
- 1.13 High level alarm
- 1.14 Alarm delay

Screen 1.2: HOME display options

- 1.21 Readout
- 1.22 Units of measurement

Screen 1.3: Bar Graph

- 1.31 Display bar graph
- 1.32 Zero point
- 1.33 Full scale
- 1.34 Scale control

Screen 1.4: Output

- 1.41 Full scale voltage
- 1.42 Zero point
- 1.43 Full scale

Screen 1.5: Serial Data Out

- 1.51 Baud rate
- 1.52 Set interval
- 1.53 Set index
- 1.54 Set format*

Screen 1.6: Miscellaneous

1.61 Backlight off time
1.62 Beeper status
1.63 Discrete sample averaging
 1.631 Pre-averaging delay
 1.632 Averaging period
1.64 Security ID check

Screen 1.7: Temperature*

1.71 Display temperature
1.72 Temperature nomenclature
1.73 Compensation format
1.74 Temperature coefficient
1.75 Reference temperature

* only appears if temperature-compensation package purchased and temperature probe plugged in

Table 4. Operational Parameters Defaults and Ranges

Operational Parameter	Default	Range
Monitor alarm	NO	YES/NO
Low level alarm	0.0	0 - 998.0
High level alarm	999.9	1 - 999.9
Alarm delay	30 (SEC)	10 - 3600 (SEC)
Units of measurement	FSU	NONE, FSU, QFT, PPM, PPB, PPT, mg/l, mg/dl, mg/ml, mg/kg, mg/g, ug/l, ug/ml, ug/kg, ug/g, ng/l, ng/ml, ng/ul, ng/kg, ng/g, ng/mg, pg/g, pg/mg, pg/ug, pg/ml, pg/ul, fg/ul, fg/mg
Display bar graph	YES	YES/NO
Graph zero point	0	0 - 9998
Graph full scale	999	1 - 9999
Bar graph scale control	MAN	AUTO/MAN
Output full scale voltage+	2V	0.1, 1, 2, 5 V
Output zero point+	0	0 - 9998
Output full scale+	999	1 - 9999
RS-232 baud rate++	9600	9600/4800
Serial data out interval++	5 (SEC)	0 - 3600 (SEC)
Serial data out index++	0	0 - 9999
Serial data out format*++	Data only	Data only/data + temp
Backlight off time	300 (SEC)	10 - 3600 (SEC)
Beeper status	ON	ON/OFF
Pre-averaging delay	15 SEC	1 - 60 SEC
Averaging period	10 SEC	2 - 60 SEC
Display temperature*	YES	YES/NO
Temperature nomenclature*	Cel	Fahr/Celsius
Compensation format*	none	none/linear/exp.
Temperature coef. (linear)*	0 %/°C /F	0 - 15 %/°C
Temperature coef. (exp.)*	0 /°C /F	0 - 15 /°C
Reference temperature (C)*	25°C	0 - 100°C
Reference temperature (F)*	77°F	32 - 212°F

+ Analog outputs can be used on Model 10-AU-005-CE with an external data logger or chart recorder (see Appendix 11A).

++ Serial outputs can be used on Model 10-AU-005-CE with an external computer or printer (see Appendix 11A).

* Visible only if equipped with the optional temperature-compensation package and the temperature probe is plugged into the instrument.

OPERATIONAL PARAMETERS DEFINITIONS

1. Screen 1.1: Alarm

a. Monitor alarm. Screen 1.11 allows the user to decide whether or not the high and low level alarms will monitor.

b. Low level alarm. Screen 1.12 allows the user to define a low level reading that will trigger an alarm.

c. High level alarm. Screen 1.13 allows the user to define a high level reading that will trigger an alarm.

d. Alarm delay. A user-settable duration for which the high or low level condition must exist before the alarm is triggered. Helps avoid mistriggering of alarms for transient conditions. Set on screen 1.14.

2. Screen 1.2: HOME display options

a. Readout. Screen 1.21 allows you to select either direct concentration or raw fluorescence readout for display on the HOME screen. If direct concentration is selected, after proper calibration, the Model 10-AU will perform all calculations relating to ranges and fluorescence signal and display the actual concentration of the sample being read.

   If you are interested in reading relative fluorescence of samples instead of actual concentration, then choose raw fluorescence data on screen 1.21.

   For both options, you can choose whether or not to have blank subtracted.

b. Units of measurement. The user may select among several different concentration units for display on the HOME and other screens. Note that these units are for display only, and have no direct correlation with concentration. Correlation with concentration is determined during calibration. If you choose to have raw fluorescence data displayed on the HOME screen, then no units will be displayed. (See screen 1.21 above.)

   NOTE: If you are using the optional internal data logging, you will not be able to access this value when data is logging. This prevents collection of erroneous data. (See Appendix 11D.)

3. Screen 1.3: Bar Graph

The bar graph is an analog display for the readout. More useful in a continuous-flow situation, it is commonly used to provide a visual comparison of concentration changes.

a. Display bar graph. Screen 1.31 allows the user to decide whether or not the bar graph will be displayed on the HOME screen.

b. Zero point. The user can set the bar graph zero point on screen 1.32. For example, if your samples are reading primarily in the 50-ppb range, you could set the bar graph zero point to 50 and full scale to 60 in order to use a larger portion of the graph. Readings outside this narrow range will not be displayed. The narrower the range, the greater the resolution.

c. Full scale. The user can set the bar graph full scale point on screen 1.33. See the example in paragraph b.

d. Scale control. Automatic or manual control of the bar graph scale can be selected on screen 1.34. In the manual mode, graph zero point and graph full scale are set by the user on screens 1.32 and 1.33. In the automatic mode, when the range is changed, the bar graph full scale will automatically become the full scale value for that range as set during calibration; the graph zero point will be zero.

4. Screen 1.4: Output

Note that the parameters on this screen are for use with external analog data logging devices and will be displayed on the Model 10-AU-005-CE even if you are not connected to an external device. They are operational only in conjunction with an external data logger or chart recorder when connected to the 10-AU through the AC or DC Power & Signal cable. (See Appendix 11A.)

5. Screen 1.5: Serial Data Out

Note that these parameters are for use with external serial data logging devices and will be displayed on the 10-AU-005-CE even if you are not connected to an external device. They are operational only in conjunction with an external computer or printer when connected to the 10-AU through the AC or DC Power & Signal cable. (See Appendix 11A for details.)

6. Screen 1.6: Miscellaneous

a. Backlight off time. On screen 1.61 the user may set the time for the backlight to be on without a keypress before it automatically goes off.

b. Beeper status. The audio beeper, which sounds when a key is pressed and when an alarm is triggered, may be turned on or off on screen 1.62.

c. Discrete sample averaging. Discrete sample averaging is a very useful feature for ensuring consistent readings on discrete samples. It allows you to average the readings over a user-settable period, and freeze the digital display for 10 seconds so you can note the reading. Thus, each sample can be read after the same amount of time has passed; and the averaging and freezing of the display minimizes both the inconvenience and potential error when readings fluctuate.

This function is particularly useful with temperature-sensitive samples. With discrete samples, correction for temperature changes is difficult to do accurately without stirring. To ensure accuracy, before taking a reading you must wait long enough for the reading to stabilize, but not long enough for temperature changes to have a significant effect. Taking a reading at a fixed time after sample insertion ensures constant temperature conditions.

To use this function, set the pre-averaging delay and the averaging period on screens 1.631 and 1.632 (see below), or you may use the default values. The default values are 15 seconds and 10 seconds, respectively.

To initiate an averaging sequence, press <*> on the HOME screen. The word "DELAY" will appear in the upper right-hand corner while the pre-delay is in effect. Then "AVE" will appear for the averaging period. When the sequence is finished, "DONE!" will be displayed and the readout will freeze for 10 seconds, displaying the averaged readout.

NOTE: If you are using the Model 10-AU with an external computer, the "frozen" reading from the HOME screen is what will be recorded during the 10-second "freeze" period.

To abort the sequence, go to another screen by pressing <ENT>, or <ESC> if "ALARM ON!" is flashing. Pressing <*> again while the averaging is taking place aborts the current sequence and starts a new one.

1. Pre-averaging delay. Once a sample is placed in the sample compartment and the light cap replaced, it takes a few seconds for the reading to stabilize. How much time depends on the sensitivity setting for the Model 10-AU and the saturation of the photomultiplier tube. The pre-averaging delay allows you to set the delay period to the minimum, but adequate, time for your readings to stabilize before the averaging period begins. Fifteen seconds is usually more than adequate. To determine the time required, place a sample in the sample compartment, replace the light cap, and see how long it takes for the reading to stabilize. Keep the light cap on between readings to avoid saturation of the photomultiplier tube.

2. Averaging period. This is the length of time the readout will be averaged. You may select an averaging period from 2 - 60 seconds. Generally, a longer averaging period helps to minimize fluctuating readings. Keep in mind, however, that the sample temperature will increase the longer it remains in the instrument, leading to possible temperature-related errors. (See the discussion about temperature in Appendix 6A.)

d. Security ID check. For security reasons, some users have found it useful to require the entry of an ID to prevent accidental changing of fluorometer settings. To enable the security ID, access screen 1.64. Once enabled, a four-digit ID will be required to access any screens other than the HOME screen. If a key is not pressed on the keypad for 30 minutes, the fluorometer will jump to the HOME screen, and an ID will have to be entered again to access any other screens.

   The 4-digit ID is a combination of the date and time as displayed on the HOME screen. Thus, it changes every minute, but you always know what it is. For example, if the time on the HOME screen is 5:14:21 PM, and the date is 9/23/91, the ID is 3241: the time in minutes (14) plus the day of the month (23), in reverse order.

7. Screen 1.7: Temperature

This will be displayed on the 10-AU-005-CE, only if the temperature-compensation package has been purchased and the temperature probe is plugged into the instrument. The temperature probe operates only when using the continuous-flow cuvette system. For a discussion of temperature and fluorescence, see Appendix 6A.

NOTE: If you are using the temperature-correction capabilities of your Model 10-AU, temperature values must be set before calibration, and YOU MUST RECALIBRATE whenever you change the temperature format or coefficient, or the reference temperature.

a. Display temperature. On screen 1.71, the user may decide whether or not temperature of the sample in the continuous-flow cuvette will be displayed on the HOME screen.

b. Temperature nomenclature. Temperature can be set to either Celsius or Fahrenheit on screen 1.72.

c. Compensation format. On screen 1.73, the user can choose none, linear, or exponential formats for the temperature coefficient. Temperature correction is correctly done with the exponential format; the linear format is included for convenience and because some researchers have used it in the past. The linear format should be used only if there is a very small temperature coefficient, as the error will escalate rapidly with increasing temperature differences.

d. Temperature coefficient. The fluorescence readings for a particular substance vary in a fixed amount with temperature. The temperature coefficient, fixed for various substances, compensates for this variance. The temperature coefficient for the analyte you are measuring can be entered on screen 1.74. See the Temperature Coefficient table in Appendix 6, page A6-1 for temperature coefficients. Be sure you select the appropriate temperature nomenclature (C/F) on screen 1.72 AND the correct compensation format (linear or exponential) on screen 1.73.

e. Reference temperature. This is the base temperature, usually room/ambient temperature, to which fluorescence readings are compared when a temperature compensation is made. Set on screen 1.75.

B. Diagnostic Information

The Model 10-AU has two diagnostic screens, 3.1 and 3.2, which contain information about the status of internal fluorometer functions and readings. (See Table 2 in Section 4, for defaults and ranges for internal fluorometer functions.)

If the readings on screen 3.1 and 3.2 are normal, it is highly probable that the fluorometer is functioning correctly.

These screens are accessed from the Main Menu by pressing <3>.

1. Screen 3.1.

Indicates the power level in percentage. Confirms **AC power** is at 115 V when reading is 100%. If your instrument is operating on **230VAC**, as long as the power level is greater than 10%, the instrument will function properly. For **DC power**, 10.2 V is 0% and 12 V is 100%. We have found that a 12-volt battery indicates approximately 70%; this is more than adequate. The **low power level alarm** will be triggered if the power level falls below 10%.

INSTRUMENT TEMPERATURE indicates the internal temperature of the fluorometer. If temperature rises above 90°C or falls below -20°C (based on internal sensor, accuracy: +/-5°C), it will trigger an alarm.

Indicates the speed in rpm of the chopper motor (which spins an optical device). It is regulated to 600 rpm, +/- 1% by internal software and hardware. If it falls below 500, an alarm is triggered.

```
Diagnostic:

Pwr level: 100%     Instr temp: XX (C)
Chopper RPM: 601 Lamp:          ON
High Volt: XXX (V)---------Within Range
Ckt status: XXXX------------------------OK
Oper hour: XXX      Oper since: xx/xx/xx
<ENT> to next screen               #3.1
```

LAMP reports whether the lamp is OFF/BAD or ON.

OPERATION HOUR shows how many hours the instrument has been operated.

OPERATION SINCE shows the earliest date at which the instrument has been operated continuously for one hour.

CIRCUIT STATUS reports a numerical value and whether it is OK or failure.The value depends on the range the fluorometer is in. In HIGH, is should be about 36; in MED about 360; in LOW about 3600.

HIGH VOLTAGE reports a numerical value and whether that value is Within Range, or Too High or Too Low. High voltage is one indication of the current operating level of the fluorometer. The number will vary: the higher the number the lower the concentration range (and typically, the greater the "noise" of the readings). High voltage will be lowest when the instrument is in the HIGH concentration range, intermediate when in the MED range, and highest in the LOW concentration range. High voltage can be increased or decreased using the Sensitivity Adjustment Knob. Once you have set the basic operating level and LOCKED the Sensitivity Knob Lock, high voltage should remain relatively stable for each of the ranges. It is a good indicator of the stability of the instrument; thus, you might want to record the high voltage level whenever you read a series of samples.

2. Screen 3.2

Output (0 to 9999.999), based on putting the fluorescence signal into the Fluorescence Readout formula below, without temperature compensation. This value is sent to digital display (rounded off to 3 digits), unless you have purchased temperature compensation, and it is activated, with the temperature probe in place; in which case, Temp. comp. readout is sent to the digital display.

This appears only if the optional temperature compensation package has been purchased and temperature probe is in place. See definition and formula in Appendix 5B, section 2b.

Output from the photomultiplier tube.

Temp. comp. readout: XXXX.XXX
Fluorescence readout: XXXX.XXX
PM signal output: XXXX.XXX

Cal std val: XXX.XX Blank: XX.XXX
FS: XX% of XXXX.XXX (PPB) at MED
Span level%: 48
<ENT> to next screen #3.2

Stored value of fluorescence signal for the standard solution set and stored on screen 2.3, Run Standard Solution, with blank subtracted (unless you set screen 2.12 to "NO").

Stored value of fluorescence signal for blank solution set and stored on screen 2.11, Run Blank. This value will be used by the instrument to calculate direct concentration or raw fluorescence (unless you set screen 2.12 to "NO"). Blank is not temperature compensated.

Full scale (FS) functions like an analog meter. It indicates the current sample's percentage of full scale reading at the current concentration range (without temperature compensation). It is also used to set the basic operating level of the instrument before operating for the first time.

Indicates the sensitivity of the instrument as set during calibration on screen 2.11, Run Blank, and screen 2.3, Run Standard Solution. Range is 0 - 99%, with a default of 48%. Span is a fine adjustment of sensitivity. Coarse adjustment is made using the Sensitivity Adjustment Knob *(details in Appendix 6B)*.

Fluorescence readout formula:

$$\frac{\text{Standard Soln Conc.} \times \text{Sample Output}}{\text{Cal std val}}$$

Fluorescence signal at a given range for current sample (range depends on the sensitivity), with blank subtracted (unless you set screen 2.12 to "NO").

Actual concentration of standard solution as entered on screen 2.2 during calibration.

Stored value of fluorescence signal for the standard solution (set and stored on screen 2.3, Run Standard Solution) with blank subtracted (unless you set screen 2.12 to "NO").

2. Screen 3.2 (continued)

a. Reading Samples on Screen 3.2. You may find it desirable or necessary to read samples with more than the three significant digits available on the HOME screen. You may do so by reading directly from screen 3.2. If you are reading direct concentration, the units you chose on screen 1.22 will be displayed. If you are interested in raw fluorescence, then "RAW" will appear next to the readout.

Calibrate the Model 10-AU following the normal procedure in Section 3F. Then, access screen 3.2, insert a sample and record the "Fluorescence readout." If you are using the optional temperature-compensation package, record the "Temp. comp. readout."

See Section 3G on Routine Operation for a discussion of auto-ranging, etc. If the readout displays "OVER," it means the concentration reads higher than full scale for the present range as currently calibrated. If you are in the Manual mode, reading on the MED range and the reading is "OVER," access screen 2.42 and change to the High range. Return to screen 3.2 to view the readout.

If the reading is OVER on the High range, then the concentration exceeds the upper limits of detectability of the Model 10-AU as currently calibrated. You might try diluting the sample 1:1 until you obtain an on-scale reading to get some idea of the concentration. Or, you can reduce the sensitivity of the instrument by recalibrating and reducing the Span level. Or, consider changing reference filters, or to a smaller cuvette size, or adding an attenuator plate. (See the discussion on decreasing sensitivity in Appendix 6A.)

Display reads ">9999". If the reading exceeds 9999.999, the maximum allowable for the screen 3.2 readout, the readout will display >9999. Access screen 2.2 and reduce the standard solution concentration by a factor of 10. I.e., if it was 500, set it to 50, or 5, if necessary to make the full scale reading less than 9999. If you do this, make note of the factor, as all of your samples will have to be multiplied by this factor to determine the actual concentration or relative fluorescence.

Minus. If the readout has a minus sign in front of it, it means that the sample is less concentrated than blank. This is more likely where you have calibrated with high blank. See Section 3E3 for what to do for high blank. It also possible that your standard or blank solutions are contaminated. If you suspect this, remake your standard and check your blank against a fresh blank or distilled water.

b. Temp. compensated readout is the value sent to the digital display (rounded off to three digits), based on putting the temperature-compensated fluorescence output for the sample and the standard solution into the formula on the Screen 3.2 page, above. (Blank is not temperature compensated.)

NOTE: Temp. comp. readout will not appear unless your instrument is equipped with temperature compensation and the temperature probe is in place. It will read the same as "fluorescence readout" if the compensation format on screen 1.73 is set to "NONE" or if the temperature coefficient is set to "0" on screen 1.74.

For the linear compensation format:

NOTE: The linear compensation format is correctly used only with small temperature coefficients. See Appendix 5A, subsection 7c.

Temperature-compensated fluorescence output =

fluorescence output x {1 + [Temp. coef. x (Sample temp. - Ref. temp.)]}

Where:

Fluorescence output is the stored value of fluorescence signal for the standard as set during calibration (Cal std val), or the fluorescence signal for the sample (Sample Output).

Temp. coef. is the temperature coefficient of the sample (item 4 in screen 1.7).

Sample temp. is the current sample temperature (C or F degrees).

Ref. temp. is the reference temperature (item 5 in screen 1.7).

Appendix 6
CALIBRATION CONSIDERATIONS

A. Calibration Considerations

Temperature. Most fluorescent materials have fairly high temperature coefficients. Usually, as temperature increases, fluorescence signal decreases. Unless corrected for, this can produce significant errors.

For this reason, the standard, the blank and the unknowns should all be read at the same temperature, or the fluorescence readings corrected for temperature.

TEMPERATURE COEFFICIENTS				
	EXPONENTIAL		LINEAR	
	/°C	/°F	%/°C	%/°F
Rhodamine WT	0.026	0.0144		
Rhodamine B	0.027	0.0150		
Fluorescein	0.0036	0.0020		
Chlorophyll (90% acetone)			0.3	0.1667
Chlorophyll (in vivo)			1.4	0.7778

Automatic Temperature Compensation. If you have purchased the optional temperature-compensation package, the 10-AU-005-CE will perform temperature correction on the standard and sample readings for you, once you have set the temperature-related values. Blank is not temperature compensated. The temperature probe operates only when using the continuous-flow cuvette.

If you have temperature-compensation **capability and the temperature probe is plugged in**, your Model 10-AU will display screen 1.7. The table above sets forth the temperature coefficients to enter on screen 1.74. Be sure you select the appropriate temperature nomenclature (C/F) on screen 1.72 AND the correct compensation format (linear or exponential) on screen 1.73.

For details on setting the temperature-related values, refer to the definitions under screen 1.7 in Appendix 5A.

NOTE: If you are using the temperature-correction capabilities of your Model 10-AU, YOU MUST RECALIBRATE if you change the temperature format or coefficient, or the reference temperature.

Temperature Considerations Using the Cuvette Holder. With the Discrete Sample Cuvette Holder, you can measure the temperature with an external thermometer and compensate for changes with the appropriate temperature-compensation formula. Or, use a waterbath or other temperature regulator and take readings for blank, standard, and samples at the same temperature. If, however, you are using a waterbath, wipe each test tube thoroughly, but quickly, with a lint-free tissue (such as Kim Wipes) before reading. Any sample read and returned to the bath should not be re-read for at least ten minutes. Even if the reading has not begun to change in the instrument (an indication that the temperature has changed), the tube will have warmed slightly and the sample temperature may increase after it is removed.

The discrete sample averaging capability of the Model 10-AU can also be used to help minimize error resulting from temperature differences. (See Appendix 5A, screen 1.63.)

Linearity and Concentration Quenching. The readout of the Model 10-AU Fluorometer is directly proportional to concentration from the smallest detectable concentration to a concentration specific to the fluorescent material, the wavelengths being used, and the path length. Above some concentration the measurements become non-linear. (See Appendix 10 for approximate linear ranges.)

As the concentration of the sample is further increased beyond linearity, the fluorometer reading rises at a decreasing rate and eventually begins to decrease, even though the concentration is still increasing. In effect, "concentration quenching" results in non-linearity.

For example, the fluorescent dyes provide linear readings from the limit of detectability of about 10 parts per trillion to about 0.1 ppm. As a rule of thumb, linearity should be checked when measuring concentrations of dye higher than 0.1 ppm, using the 25 mm Continuous-Flow Cuvette or 25 x 150 mm Cuvette Holder. (For other cuvettes, refer to the instructions accompanying them.)

At dye concentrations below 0.1 ppm, a single-point calibration (one standard and a blank) may be used to calibrate the Model 10-AU. For concentrations between 0.1 and 0.5 ppm, a multi-point calibration curve (using multiple concentrations of the standard) must be prepared, or the samples must be diluted and the reading obtained multiplied by the dilution factor. (Much above 0.3 parts per million, dilution will be more accurate.) Above 0.5 ppm, "concentration quenching" occurs, and samples should be diluted into the linear range before taking readings.

Linearity may be checked by diluting a sample 1:1 or some other convenient ratio. If it is linear, the reading will decrease in direct proportion to the dilution. If the reading goes up ("concentration quenching"), you are beyond the range for a calibration curve. If the reading does not decrease in direct proportion to the dilution, you are in the range for a calibration curve, but beyond the linear range.

Changing Applications. You set the basic sensitivity of the Model 10-AU using the Sensitivity Adjustment Knob before calibrating for the first time. If you change the lamp, filters, or cuvette size, you will have to readjust the Sensitivity Adjustment Knob. See Appendix 6B below. DO NOT ADJUST the Sensitivity Adjustment Knob otherwise. It is not necessary and it will disrupt your study.

Calibrating With the Continuous-Flow System

The 25 mm Injector Flow Cell is designed with a valve and Luer-lock injection port which allows injection of the standard and blank directly into the flow cell.

If the instrument does not have the Injector Flow Cell, then accurate calibration can be made by setting up the 10-AU so that the continuous-flow cuvette may be poured full of the standard and blank. Several flushings are required to eliminate any trace of the preceding sample. To ensure complete filling of the cuvette without having any large bubbles trapped, it is best to fill through the lower Intake Fitting. Significant air bubbles will greatly affect the accuracy of your readings. This method must be used when working with the 3 mm or the 1 mm flow cell.

However, when calibrating with the flow-through method, it is difficult to know the sample temperature. If you have purchased the optional temperature-compensation capability, this will simplify calibration using the flow-through method. (See Appendix 5A, screen 1.7.) If you have not, and temperature compensation is important to your study, note that simply measuring the temperature prior to filling the cuvette is not sufficient. The cell itself may be warm and will quickly warm the sample. It is best to pump the standard through the cell and measure it while it is flowing.

Alternately, you could prepare a valving system in which standard, blank, and unknown are pumped through the fluorometer in identical fashion. Such a system could include a means of stopping flow, to conserve on standard. If you do stop flow, do it only briefly as the instrument will warm the solution.

Note that with a few exceptions, the flow rate through the Continuous-Flow Cuvette System is not important and is at the operator's discretion. (See Appendix 4B, Flow Rate.)

You CANNOT calibrate the 10-AU by installing the Discrete Sample Cuvette Holder (25 x 150 mm), calibrating the instrument, then switching back to the continuous-flow system. The systems have a slightly different calibration factor and results will not be accurate. Note that you MAY set the basic operating level of your instrument using the discrete sample holder (as set forth in Appendix 6B, following). The difference between the two systems is not significant for this procedure; and after proper calibration your results will be accurate.

Cuvettes. Cuvettes must be clean and dry on the outside. Take particular care not to contaminate your samples, especially if working with low concentrations.

If quartz cuvettes are used, rinse them thoroughly before reusing. If possible, read samples progressively, from low to high concentrations; you will achieve more accurate readings, and contamination is not as significant.

Averaging Readings. When working with test tube samples, keep in mind that there can be slight variations from tube to tube. For this reason, it is common to have three (or more) tubes of standard and to average the readings. You may also use replicate samples as well. It is for you to determine how important the reading of one sample is.

Bubbles in the Sample. The presence of bubbles in your sample, even minute ones, will affect your readings, producing erratic or fluctuating readings. If bubbles occur in test tube samples, you can wait for them to settle; or cap the test tube and rotate it gently from side to side and up and down.

In continuous-flow systems, if bubbles are present, check your delivery system for leaks. Make sure the pump you have chosen is the best for your study. (See Appendix 4B.)

Storage of samples. The storage material must be compatible with the solvent and must not adsorb or affect the sample. Borosilicate glass is usually acceptable for all samples. Ordinary glass sometimes has outcroppings of soda lime, which can affect pH in poorly buffered samples. Fluorescence is generally more sensitive to pH than is absorption spectroscopy. The fluorescence of Rhodamine WT, for example, is relatively consistent between 4 and 10.5, but drops sharply beyond these extremes.

Increasing Sensitivity. Sensitivity can be increased using the Sensitivity Adjustment Knob. (See Appendix 6B.)

Sensitivity can also be increased by using a larger cuvette size. If you have increased sensitivity to its maximum using the Sensitivity Adjustment Knob, and have tried reading samples on the Low concentration range, with Span close to 100%, and find you could still use more sensitivity, switching to a larger cuvette size will help. (See Appendix 10.)

Sensitivity increases with increasing cuvette size: the 25 mm cuvette is the most sensitive, then the 10 mm (analogous to the 13 x 100 mm Cuvette Holder), and finally the 3 mm Continuous-Flow Cuvette. (See the Turner Designs Model 10-AU Digital Fluorometer Ordering Information booklet.)

Sensitivity may be increased by a factor of 10 by installing a 1 ND (neutral density) reference filter. A 2 ND reference filter will increase sensitivity by a factor of 100. (For information on filter installation, see Appendix 8.)

Decreasing Sensitivity. Sensitivity can be decreased using the Sensitivity Adjustment Knob. (See Appendix 6B.)

Sensitivity can also be decreased by using a smaller cuvette size (10 mm, 3 mm, or 1 mm by special order). If you have decreased sensitivity to the minimum using the Sensitivity Adjustment Knob, and have tried reading samples on the High concentration range, with Span close to 0%, and find you would still like to reduce sensitivity, switching to a smaller cuvette size will help. (Appendix 10.)

Adding an attenuator plate to the excitation filter holder can also reduce sensitivity. There are two sizes: the 1/4" attenuator, which reduces sensitivity by a factor of 5; and the 1/16" attenuator plate, which reduces sensitivity 75-fold. (See Appendices 8 and 9.)

If a 1 ND or 2 ND reference filter is installed, removing it will decrease sensitivity by a factor of 10 for the 1 ND and 100 for the 2 ND. (See Appendix 8 for removal instructions.)

B. Setting the Basic Operating Level Using the Sensitivity Adjustment Knob

1. Introduction

You must set the basic operating level (sensitivity) of your Model 10-AU using the Sensitivity Adjustment Knob BEFORE calibrating your instrument for the first time. Thereafter, it will not be necessary to readjust the Sensitivity Adjustment Knob unless you change to a different cuvette size or a different kind of lamp or filters; or unless you want to significantly increase or decrease sensitivity. DO NOT ADJUST the Sensitivity Adjustment Knob otherwise. It is not necessary and it will disrupt your study. If you adjust the Sensitivity Adjustment Knob, you must recalibrate the instrument or your readings will be inaccurate.

The Model 10-AU is extremely sensitive and in most applications can read a broad range of concentrations with great accuracy. After setting the operating level using the Sensitivity Adjustment Knob, the instrument will have a range of about 5,000,000 to 1 (i.e., 500 ppm to 0.1 ppb) using only the Span and concentration ranges to adjust sensitivity.

The operating level will be set on screen 3.2 by adjusting the FS% using the Sensitivity Adjustment Knob. Operating level will be adjusted with the calibration settings in the default position (reset to default on screen 2.6).

You will need a standard concentration that is approximately 50% of the maximum concentration you wish to read. Make sure the maximum selected is within the linear range for your substance (see Appendix 6A). (See the sections below for details about Rhodamine WT or chlorophyll.) Note that the higher the concentration you choose to read, the more resolution you will sacrifice when reading low samples. Thus, be reasonable when considering your maximum. The broader the range of concentrations you are trying to read, the greater the loss of resolution on the low end.

If you are interested in reading very low concentrations (close to the minimum levels of detectability), you should also have a very low standard concentration on hand to verify that you can distinguish it from blank. (See Appendix 10.)

Sensitivity of the Model 10-AU is at maximum when the instrument is in the LOW range, Span at 100%, and the Sensitivity Knob is fully clockwise.

Feel free to experiment with various Sensitivity Adjustment Knob settings to find one that works for you. You can't hurt the instrument by changing the Knob setting, though you should do your experimenting before you begin your actual study, as **changing the Sensitivity Knob requires recalibration** and readings on one sensitivity setting do not necessarily compare exactly with readings on a different setting. (But see Appendix 6C for the procedure for retrieving a former sensitivity setting.)

2. Setting the Sensitivity Adjustment Knob

This is a *general method* for setting the operating level. It should allow you to read the maximum concentration you selected and give you adequate sensitivity for low concentrations--assuming the maximum you selected is within the linear range for the instrument.

Please see the sections below on Rhodamine and chlorophyll for suggestions specific to those applications.

a. Prepare or obtain a calibration standard solution that is about 50% of the maximum concentration (within the linear range) you wish to read.

b. Turn on the fluorometer and allow it to warm up for at least 10 minutes.

c. Access screen 2.43 and set the concentration range control to MAN.

d. On screen 2.42, set the instrument to the MED concentration range. (For certain studies, e.g., *in vivo* chlorophyll or oil, you may use the LOW or HIGH ranges, respectively.)

e. On screen 2.6, reset the calibration values to their default values. At default the values are: 1. Blank reads zero; 2. Cal std val reads 50.000; 3. Standard solution concentration reads 15.000; Span reads 48%; and maximum for the Full Scale Value Table reads 900 for High, 90 for MED, and 9 for Low.

f. Access screen 3.2. The third line from the bottom will read: FS%: XX% of 90.000 units at MED. The full scale (FS) reading is dependent upon the concentration of any sample in the sample compartment and the current sensitivity adjustment.

g. Unlock the Sensitivity Adjustment Lock with an Allen wrench (see Section 2B, Figure 1, for location).

h. Fill the clean flow cell or a cuvette with the calibration standard solution.

i. Adjust the Sensitivity Adjustment Knob (the large recessed knob located at the lower right of the keypad; see Figure 1, Section 2B) until the FS% (third line from the bottom) reads approximately 40-60%; i.e., "FS%: 55% of 90.000 units at MED." It is not necessary to be exact.

Turning the Sensitivity Knob clockwise increases sensitivity (FS%); turning it counterclockwise decreases sensitivity (FS%).

The Sensitivity Adjustment Knob is very responsive and even a small adjustment can cause a great change in readings. Thus, the proper way to adjust the Sensitivity Adjustment Knob is to turn it very slightly (using a coin), then pause until the reading reaches equilibrium, then adjust, pause, etc., until the desired reading is obtained.

There is a clutch mechanism that prevents damage if the control is turned beyond its maximum and minimum ranges. When you have reached the maximum or minimum, the FS% will not change, even though you turn the control.

On screen 2.5, the Time Constant can be set to 1, 2, 4, or 8 seconds. Setting the Time Constant to 1 or 2 seconds will provide a faster response time.

<u>If the FS% is less than 10% and barely changes</u> no matter which direction you adjust the Sensitivity Knob, make sure the Sensitivity Adjustment Lock is unlocked. Then, check to make sure that the calibration standard solution you are using is an accurate dilution. If the FS% still barely changes when the Sensitivity Knob is turned, it is likely that the range selected is not sensitive enough for the application and sample you are trying to read. If you are in the MED range, go to screen 2.42 and change to the LOW range (if in HIGH, change to MED). Return to screen 3.2 and adjust the Knob until the FS% reads 40-60% of MED or 40-60% of LOW.

If you cannot obtain adequate sensitivity in the LOW range, refer to the procedure for reading very low concentrations, in the Chlorophyll section below, and to Appendix 6A, for methods for further increasing sensitivity.

<u>If the FS% on the HIGH range reads greater than 111%</u> and will not go below 100% with the Sensitivity Knob fully counter-clockwise, the concentration is too high to be read by the

instrument at the current sensitivity levels. You can dilute the calibration standard solution until the FS% can be adjusted to less than 100% (this will lower the maximum concentration you can read). Or, see Appendix 6A for ways of further decreasing sensitivity.

To find the maximum concentration (full scale) you will be able to read at the current settings, after the FS% is set on screen 3.2 (assuming FS% was set with Span at default of 48%):

Maximum concentration (FS) =

$$\frac{\text{Concentration of calibration standard x 500}}{\text{FS\%}}$$

Range FS% was set on	**FS on HIGH**	**FS on MED**	**FS on LOW**
HIGH	FS	FS ¸ 10	FS ¸ 100
MED	FS x 10	FS	FS ¸ 10
LOW	FS x 100	FS x 10	FS

NOTE: FS (maximum concentration) is NOT the same as FS%.

j. When the desired reading is obtained, LOCK the Sensitivity Adjustment Lock by turning it clockwise with the Allen wrench. The basic sensitivity is now set, and you will use the Span and concentration ranges during calibration to set sensitivity for your study.

k. Press <ESC> to get out of screen 3.2.

l. Calibrate your Model 10-AU following the normal procedure. (See Section 3F.)

3. Setting the Sensitivity Adjustment Knob for Rhodamine

Using the 25 mm cuvette, Rhodamine WT (and B) readings are linear to approximately 100 parts per billion--active ingredient. (Rhodamine WT usually comes as a solution of about 20% active ingredient. Thus, if dilutions were made treating the dye as 100% tracer, the resulting dilutions would be linear to 500 ppb.) We have found that the 10-AU provides satisfactory readings for most studies when the Sensitivity Adjustment Knob is set using the concentrations, range, and FS% in Table 5. However, please note that the FS% is a rough sensitivity adjustment. It is not necessary for satisfactory operation to achieve an FS% within the exact recommended range.

Follow the procedure in subsection 2 above, referring to Table 5 for appropriate settings. For example, if you are using a 20 ppb (active ingredient) concentration, in step d you will select the MED range, and in step i you will set the FS% to 70-90%.

For instructions for preparing dye standards, ask us for our monograph: "Preparation of Standards for Dye Studies Using Rhodamine WT."

TABLE 5. Settings for Rhodamine

Rhodamine[1]	**Cuvette**	**Conc. to use**	**FS%**	**Range**
20% active ingredient	25 mm	20 ppb	70-90[2]	MED
As 100% tracer	25 mm	100 ppb	70-90[2]	MED

1 Rhodamine WT is often supplied as a 20% aqueous solution, i.e., as 20% active ingredient.

2 Please note that this is a very flexible setting. If, after setting the instrument to this level, you find that OVER appears frequently when reading samples, then reset basic sensitivity on the HIGH range to an FS% of 30-50%. If superior resolution for low concentrations is desired, then use a low concentration standard (2 - 5 ppb) and set FS% to 70-90% on the LOW range.

4. <u>Setting the Sensitivity Adjustment Knob for Chlorophyll</u>

a. Extractive Methods

Most researchers will use the 13 mm cuvette for extracted chlorophyll.

Using the 13 mm cuvette, the US Environmental Protection Agency has found detection limits of 0.05 μg/L (ppb; or 50 parts per trillion); the upper limit of linearity was 250 μg/L. (For Method 445.0, contact Turner Designs for Method 445.0, "*In Vitro* Determination of Chlorophyll *a* and Pheophytin *a* in Marine and Freshwater Phytoplankton by Fluorescence." Or, refer to our website: http://www.turnerdesigns.com/applications/998_6000.htm or the U.S.E.P.A. website: http://www.epa.gov/nerlcwww/marinmet.htm)

Contact Turner Designs about ready-to-use chlorophyll standards in 90% acetone and stable solid secondary standards.

We have found that the Model 10-AU provides accurate readings when the Sensitivity Adjustment Knob is set using the concentrations, range, and FS% in Table 6.

Follow the procedure in subsection 2 above, referring to Table 6 for appropriate settings. For example, using a 150 ppb standard, in step d you will select the HIGH range, and in step i you will set the FS% to 30-50%.

<u>If you cannot obtain the recommended FS%</u>, try installing a 2 ND reference filter. (See Appendix 8 for instructions.) This will increase the sensitivity by a factor of 100.

<u>If you can't reduce the FS% to 100 or below</u>, check to see which reference filter is in place. If it is a 2 ND, replace it with a 1 ND <u>or</u> simply remove the 2 ND filter and set sensitivity without a reference filter. (See Appendix 8 for replacement instructions.)

b. *In vivo* Continuous-Flow Method

The Model 10-AU is often used aboard ship with the 25 mm continuous-flow cuvette. To set the basic operating level, follow the procedure in subsection 2 above, referring to Table 6 for appropriate settings. For example, in step d go to the MED range. While sample lake or marine water is flowing through the instrument, in step i, set the FS% to approximately 30-80%. If FS%

is greater than 111% on the MED range, switch to the HIGH range and set the FS% to 30-80%. If you can't reach the desired FS% on the MED range, switch to the LOW range and set to 30-80%.

Note that in most cases you will be setting the basic operating level with an unknown sample from the body of water you are investigating. During the calibration procedure while running the standard on screen 2.3, you should take a grab sample of the water immediately after it passes through the flow cell for later extraction to determine actual chlorophyll concentration. You will then use a ratio method to compare all other readings with the standard. (See Section 3G, Routine Operation.)

NOTE: For *in vivo* chlorophyll studies in natural systems (where sample water flows through the instrument's flow cell without pretreatment), the fluorometer reading is NOT the actual concentration of chlorophyll present. It is, however, directly proportional to actual concentration. The actual concentrations of chlorophyll will be determined later by comparing instrument readings to a known concentration of your sample determined using an extractive method such as EPA Method 445.0. The *in vivo* method greatly reduces the number of samples that must be extracted, providing a reliable "map" of chlorophyll concentrations where large numbers of samples are to be read.

c. Reading Low Concentrations *in vivo* or Extracted

Chlorophyll can be detected as low as 10 parts per trillion using the Model 10-AU. Some researchers have reported detecting single cells. Detectability levels are, however, highly species dependent and affected by the health and development of the particular organism.

To obtain maximum sensitivity levels, use the 25 mm cuvette. Install a 2 ND reference filter (see Appendix 8).

Take a very low sample, say 50 parts per trillion (0.05 μg/L).

Follow the procedure in subsection 2 above, referring to Table 6 below for recommended settings. For example, in step d go to the LOW range. Gently swirl the cuvette before inserting into the instrument (step h). Adjust the FS% in step i to 5-25%, with 25% preferred. Note the PM signal output (screen 3.2). Some fluctuation should be expected as the instrument is operating at a very high level of sensitivity.

Remain on screen 3.2. Put in a clean 25 mm cuvette containing a blank solution. Note the FS% and the PM signal output and compare it to the FS% and the PM signal output for the 0.05 μg/L sample. Is the sample reading distinguishable from the blank?

If not, it might help to install the 10-318 (1/4") attenuator plate (see Appendix 8 for instructions). With this plate, you will be able to turn up the sensitivity and may find better resolution of low samples as compared to blank. If this doesn't help, it probably means your particular sample is not detectable by the Model 10-AU at that low a concentration.

TABLE 6. Settings for Chlorophyll

Chlorophyll	**Cuvette**		**Conc. to use**[2]	**FS%**[3]	**Range**
Extracted[1]	13 mm		150-200 μg/L	30-50	HIGH
"	"	<u>or</u>	15 - 20 μg/L	30-50	MED
"	"	<u>or</u>	2 - 5 μg/L	70-90	LOW
<u>In vivo</u> (flow)	13 mm		unknown	30-80	MED or LOW
Low concentration	25 mm		0.05 μg/L	5-25	LOW

1 For extracted chlorophyll, you can use either the low or the high concentration standard (prepared chlorophyll *a* standards in 90% acetone available from Turner Designs).

2 These concentrations need not be exact, as readings will be adjusted during calibration. The idea is to obtain a satisfactory sensitivity level. These are not the only concentrations you can use; they are to give you basic recommendations as to concentration, range, and FS%.

3 Please note that this is a very flexible setting. If, after setting the instrument to this level, you find that OVER appears frequently on the HIGH range when reading samples, then reset basic sensitivity to a lower FS%. If the best resolution for low concentrations is desired, see subsection C, above.

C. Sensitivity Setting Retrieval

It is possible to retrieve a previous sensitivity setting on the Model 10-AU. To do so, three settings must be noted during calibration. In addition, <u>the same</u> filters and lamp must be used, installed in exactly the same position as for the previous study. The same size cuvette must also be used. A retrieval can be done with an error of less than 0.5% <u>if</u> the Sensitivity Adjustment Knob has not been changed.

If the Sensitivity Adjustment Knob has been changed, the characteristics of your instrument's photomultiplier tube, the time elapsed between studies, and the operating temperature will affect the accuracy of the retrieval. For a fairly accurate retrieval, perform the retrieval procedure no more than two weeks after the former study, at the same ambient temperature, after the instrument has been operating for the same amount of time as before.

To retrieve a former sensitivity setting, you must have made note of the following three factors during the calibration for the previous study:

1. Concentration range in which you calibrated (item 4 on screen 2.0);
2. Span percentage on screen 2.3 (also visible on screen 3.2);
3. High voltage at the concentration range in which you calibrated (screen 3.1).

In addition, mark the lamp and filters and their precise locations if you remove them. If you are readjusting the Sensitivity Adjustment Knob, for a more accurate retrieval note the time of operation and ambient temperature.

To retrieve a previous setting:

1. Install the same lamp and filters (in the same holders) in the same positions (same direction, facing the same way) as in the former study. In addition, use the same size cuvette or flow cell.

2. Allow instrument to warm up for at least 10 minutes. If the Sensitivity Knob has been adjusted, let the instrument run for the same amount of time as before at roughly the same ambient temperature.

3. Access screen 2.42 and set range to former setting.

4. Access screen 3.1 and check High Volt. If you have not readjusted the Sensitivity Adjustment Knob, it should be close to the former reading. If it matches the former reading, then go to step 5.

   If it does not match, then unlock the Sensitivity Adjustment Lock using an Allen wrench. Adjust the Sensitivity Adjustment Knob slowly, pausing between adjustments until the High Voltage matches the previous setting. Then lock the Sensitivity Adjustment Lock.

5. Recalibrate your instrument following instructions in Section 3F, <u>except</u> that when you are running your standard on screen 2.3, **set Span to the previous percentage**. Be sure to press <*> to save setting.

Appendix 7
SAMPLE SYSTEM: MAINTENANCE & INSTALLATION

The sample system of the Model 10-AU-005-CE will operate with either a cuvette holder for discrete samples, or with a flow cell for continuous or on-line sampling.

The cuvette or flow cell is made of glass or quartz, depending upon the application. (See Appendix 9.)

The 25 mm Continuous-Flow Cuvette is standard with your fluorometer, unless you requested otherwise. The cuvette is made of borosilicate glass. The system has a pressure rating of 25 psig, with Intake and Exhaust Fittings of 1/2" female pipe thread.

Cuvette holders and flow cells come in various sizes. Generally, the larger the cuvette or flow cell, the lower the concentrations that can be read. Thus, for the greatest sensitivity, you would use the largest cuvette or flow cell (25 mm). To read higher concentrations, change to a smaller diameter flow cell. The shorter path length increases the linear range and reduces the effects of light-absorbing materials. (See Appendix 10; consult the Turner Designs Model 10-AU Digital Fluorometer Ordering Information booklet.)

Cuvette holders and continuous-flow cuvettes are available in the following sizes:

Cuvette Holders:	25 mm, 13 mm
Continuous-Flow:	25 mm (One-Piece Flow Cell or 3-piece for applications where particulate material may cause clogging); 10 mm, 3 mm, or 1 mm (special order only)

Refer to the Turner Designs Model 10-AU Digital Fluorometer Ordering Information booklet for more information about specific set-ups.

CAUTION Components of the Flow Cell (Continuous-Flow Cuvette System) are made of PVC, Delrin, and/or nickel-plated brass; and the seals are made of elastomers suitable for use in freshwater and marine environments. When using the Continuous-Flow Cuvette System, DO NOT use organic solvents such as acetone, methanol, or pyridine, or corrosive materials such as strong acids and bases in the flow cell.

A. Cuvette Holder Maintenance

Generally, the only maintenance required for the Model 10-AU-005-CE cuvette holder system is to keep the sample compartment clean and dry.

NOTE: Insert clean test tubes or cuvettes, completely dry on the outside, and free of dirt, oil, or lint. The proper size **must be** used (i.e., 25 x 100 or 150 mm; 13 x 100 mm).

Check periodically for **moisture** in the Sample Compartment. When not in use, keep the Light Cap on to keep out dirt and moisture.

Condensation forming on the outside of the cuvette can cause drifting readings. If there is a sufficient volume of air to pull moisture from, it can also cause erratic readings as droplets break free and run down. For this reason, keep the samples at a temperature that avoids condensation.

NOTE: The 10-AU-005-CE's electronics are sealed so that they are protected from damage or hazard in case of a spill inside the sample compartment. In addition, the lamp compartment is separated from the sample compartment to prevent damage to lamp components in case of a spill or leak. There is also a drain in the bottom of the cuvette holder. However, it is best to avoid spills or leaks. See subsection G, below, for the procedure to follow if water enters the Sample Compartment.

Storage. To store your fluorometer with the cuvette holder in place, open the sample compartment and make sure it is clean and dry. If you are storing it for an extended period, you might want to add a few desiccant packets (see the Turner Designs Model 10-AU Digital Fluorometer Ordering Information booklet), although it is not essential. Put the Light Cap on and tape it securely in place.

B. Continuous-Flow Cuvette Maintenance and Leaks

As long as the cuvette appears visually clean, and there is no sign of leakage, maintenance is not required. If the fluid lines leading to the system are moved or stressed, check for leaks!

The One-Piece Flow Cell can be cleaned easily by removing the plug at the top of the flow cell and **GENTLY (avoid scratches)** brushing the inside of the glass cuvette with the flow cell brush.

If you do not have the clean-out flow cell, then the flow cell may be inspected by going through steps 1 - 4 in subsection E, below. To remove it for cleaning, drain the system, remove external connections, then continue with subsection E.

The flow cell cuvette is borosilicate glass (or quartz in some applications), and may be cleaned by normal techniques.

The O-rings are a Nitrile (Buna N) rubber. Chemical resistance is good. They must be lubricated before reassembly. See subsection F.

The Intake and Exhaust Fittings are nickel-plated brass. Do not use strong chemical cleaning agents. (See "CAUTION" previous page.)

C. Flow Cell: Condensation and Desiccant Use

Condensation forming on the outside of the cuvette can cause drifting readings as it builds up. If there is a sufficient volume of air to pull moisture from, it can also cause erratic readings as droplets break free and run down.

For this reason, the entire sample area is gasketed and sealed, and a space for desiccant is provided.

Usually, you won't have any problem, even without desiccant, since the free volume of air inside the sample area is small and there isn't much moisture to condense out.

If problems are encountered, proceed as follows, referring to Figure A2 (Appendix 8):

1. Remove the Sample Compartment Cover (8 hex screws).
2. Remove and discard any spent desiccant.
3. Remove the tape seal from the bottle of desiccant packages supplied with your instrument. Remove two packages, close and re-seal the bottle.
4. Place the packages in the position shown in Figure A2.
5. Re-install the Sample Compartment Cover with a minimum of delay. All 8 screws should be installed loosely - to allow best alignment. Then tighten progressively until all screws are snug, but not dead tight. (Over-tightening can cause distortion of the cover and leakage.)
6. Order a Desiccant Replacement Kit (bottle of ten), if needed.

D. Flow Cell External Connections

If your instrument is equipped with the 3-piece flow cell, then, as received, your instrument has both the Intake and the Exhaust Fittings installed so that their threads face forward. If it makes your plumbing job easier, either or both of these fittings may be set so they face left. See subsection F below. Intermediate positions are <u>not</u> available. Pull the fittings out <u>before</u> rotating them.

If your instrument is equipped with the One-Piece Flow Cell installed, it is ready for external connections. For installation instructions, refer to PN 998-2468 at the end of this chapter.

The following points should be considered, when making external connections:

1. The 25 mm flow cell fittings accept standard 1/2" NPT male (some fittings accept female, so make a visual check) pipe threads. (See the Turner Designs Model 10-AU Digital Fluorometer Ordering Information booklet for specifications for other flow cells.) Pipe dope or the plastic tape sealers will be required.

CAUTION

2. Don't over-tighten! It is possible to break the solder joint in the fittings - and also, with extreme force, you could distort the fittings to the point where the rubber-cushioned cuvette will break.

   We do not recommend rigid pipe hook-up. Stress in the field may weaken connections, resulting in damage to the flow cell or leaks.

3. After you have attached plumbing connections to the 10-AU, remove the Sample Compartment cover and make sure the Upper Set Screw and the Lower Set Screw holding the flow cell in position are still tight. (DO NOT overtighten!).

CAUTION

During measurements, provide a strain relief for the intake and exhaust hoses to avoid loosening these fittings.

4. Normally, you'll be going to a hose on both the Exhaust and Intake fitting. This hose must be opaque for the first several feet, at least. If not, light can "leak" in and upset your measurements.

   If you have any doubts about light leakage, shade the hose, and see if the reading changes. Select the most sensitive instrument range that you plan to use.

5. If you have any question about flow cell cuvette breakage or leaks, check visually for breaks, then turn the sample system on and check for leaks. See subsection B.

6. Air bubbles will cause erratic readings. The packing gland of a pump is often a source of inward air leakage. This problem is often cured by putting the sample pump on the exhaust end of the system, so it pulls sample through rather than pushes it through.

CAUTION

7. Remember that the 25 mm continuous-flow system is rated at 25 psig! If, for example, you are using a pressurized system to combat dissolved gas release, etc., be sure you do not exceed this rating.

E. Removing the Cuvette Holder or Three-piece Flow Cell

For the One-Piece Flow Cell, reverse the installation instructions (PN 998-2468) at the end of this Appendix.

1. Turn off the power and drain the system! If present, remove all connections from the Intake and Exhaust Fittings.

2. It is not necessary to remove the filters, however, for details about filter removal and changing, refer to Appendix 8.

3. Remove the 8 hex screws that retain the Sample Compartment Cover and pull cover toward you (Excitation Filter Holder will remain with cover). If the gasket under this cover sticks, remember that it is glued to the cover. Inserting a thin knife gently between the gasket and the Sample Compartment Casting should be sufficient.

   See Figure A2 for locations of various parts with the Sample Compartment Cover removed.

4. If you have just received your instrument, the lamp will be installed. It is not necessary to remove the lamp, however, to change lamp, see Appendix 8 for instructions.

Ultraviolet Light. In some applications such as Rhodamine WT and Short Wavelength Oil, the light source is supplied by a clear quartz lamp, which is a source of ultraviolet light. This light can cause permanent damage to the eyes if observed. The lamp is contained in the sample compartment, which prevents any hazard during normal operation. When the sample compartment cover is removed for any reason, a safety interlock switch turns the lamp off. **DO NOT** override this safety switch. You must wear approved protective goggles whenever there is a potential for exposure to ultraviolet light from the quartz lamp. The instrument should be turned off and unplugged before removing or changing the lamp.

4. Cuvette Holder Removal

   a. Refer to steps 1 - 4.

   b. Using the Allen wrench, loosen the Upper Set Screw and the Lower Set Screw.

   c. If the 10-AU-030 13 & 25 mm Cuvette Holder Set (2-piece), push the cuvette holder out through the opening in the top of the sample compartment.

Note: With the 10-AU-030 13 & 25 mm set, if you are simply changing from 25 mm discrete samples to 13 mm discrete samples, or vice versa, then the 13 mm holder slips inside the 25 mm holder. To ensure proper alignment, the metal pin on the top lip of the 13 mm portion fits into the groove on the top lip of the 25 mm body.

Note: If you are changing to the 10 mm square cuvette holder, then the 10 mm holder slips inside the 25 mm holder. To ensure proper alignment, the metal pin on the top lip of the 10 mm portion fits into the groove on the top lip of the 25 mm body.

d. Wipe up any spilled liquids.

5. <u>One-Piece Flow Cell Removal</u>

Refer to installation instructions (PN 998-2468) at the end of this Appendix. Reverse the procedure.

6. <u>Flow Cell (25 mm 3-part) Removal</u>

a. Refer to steps 1 - 4.

b. Using the Allen wrench, loosen the Upper Set Screw and pull up on the Exhaust Fitting, rotating it back and forth a little, to free it. Be careful! The flow cell cuvette may come out with the Exhaust Fitting. If it does, remove it with the Exhaust Fitting. If not, pull it up and out after you have removed the Exhaust Fitting.

c. Loosen the Lower Set Screw. Pull down on the Intake Fitting, rotating it back and forth a little, to free it.

<u>NOTE</u>: The Intake and Exhaust Fittings are identical. Don't worry about getting them mixed up.

d. Wipe up any spilled liquids. If any old desiccant bags are in place, remove and discard them.

F. Cuvette Holder or Flow Cell Installation

The installation of cuvette holder or flow cell will start with the following items removed from your instrument (see subsection E):

1. Sample Compartment Cover, and 8 screws.
2. Cuvette Holder or Flow Cell

Begin with step 1 through 3, below, depending on the sample system you wish to install.

1. <u>10-AU-030 13 & 25 mm Cuvette Holder Set Installation; 10 mm Square Cuvette Installation</u>

   Note: With the cuvette holder, when taking readings, the Light Cap must cover the opening in the top of the Sample Compartment and any test tube samples to get accurate readings.

   a. Locate the larger diameter long cylinder. Note that it has an o-ring on each end to ensure a snug fit. If necessary, lubricant the o-rings with a small amount of silicone lubricant.

   Orient the holder so the rounded side is facing to the left, farthest from the lamp. Insert it in the top opening of the sample compartment until the upper lip is flush with the top opening. Center the oval slits from top to bottom inside the compartment, and make sure the front face is precisely parallel to the front of the instrument. One oval slit should be precisely parallel to the lamp, and another parallel to the back of the instrument.

   CAUTION

   b. Tighten the Lower Set Screw <u>only</u> so it is snug, but do not over-tighten. <u>DO NOT TIGHTEN</u> the Upper Set Screw; it may damage the holder. Just tighten it enough to clear the sample compartment cover and leave it loosely in place.

   c. For the 10-AU-030 13 & 25 mm holder set, to change to the 13 mm holder, simply insert the smaller diameter piece inside the larger piece, and align it by slipping the metal pin on lip of the 13 mm portion into the groove on the lip of the 25 mm portion.

   Note: If you are changing to the 10 mm square cuvette holder, then the 10 mm holder slips inside the 25 mm holder. To ensure proper alignment, the metal pin on the top lip of the 10 mm portion fits into the groove on the top lip of the 25 mm body.

   d. Go to steps 4 - 8, below.

2. One Piece Flow Cell Installation

   For the One-Piece Flow Cell, refer to installation instructions (PN 998-2468) at the end of this Appendix.

   Then, go to steps 4 - 8, below.

3. Flow Cell (25 mm 3-piece) Installation

   a. Carefully inspect the two O-rings on the Exhaust Fitting and two on the Intake Fitting for nicks or tears. If there is any sign of deterioration, replace them with new O-rings, found in your Cuvette Replacement Kit. (This kit was supplied with your instrument.)

      If you do replace the O-rings, order a replacement O-Ring Kit.

      Be sure that the round rubber flat washers supplied on the Exhaust and Intake Fittings are in place. These flat washers are near the smaller O-rings, against the flat surface on the fittings that makes contact with the end of the cuvette.

CAUTION These flat washers should be removed when the Continuous-Flow Nephelometry Kit is installed.

   b. Lubricate the O-rings. You'll find excess lubricant with the O-rings in your Cuvette Replacement Kit. Silicone oils will also do.

   c. Work the Intake Fitting up into position. The threads can face toward you, or to the left, but not at an intermediate angle. Tighten the Lower Set Screw.

   d. Carefully clean the glass or quartz cuvette. Slip it down through the hole where the Exhaust Fitting will eventually go, and press it into place on the Intake Fitting. The plastic handle of a screwdriver may be used to push the cuvette into place.

   e. Work the Exhaust Fitting down until it almost engages the cuvette. Be sure that the cuvette is properly aligned with the Exhaust Fitting. Push the Exhaust Fitting down, to engage the cuvette fully.

      Note that the Exhaust Fitting may also face toward you or face to the left, but may not be at an intermediate angle.

   f. Loosen the Lower Set Screw on the Intake Fitting.

Adjust the fittings up and down until the cuvette is centered.

The ends of the cuvette should contact the flat rubber washers described in paragraph a, above, but should not compress them. (The liquid seal is supplied by the O-rings. The flat rubber washers protect the cuvette from direct contact with the metal Exhaust and Intake Fittings.)

g. Rotate the Fittings a little as you slowly tighten the set screws - so the set screws are centered on the flats on the fittings. Wipe any fingerprints off the cuvette.

   If you have any questions about leakage, now is the time to hook up the external connections, and make a visual check.

h. Wipe up any spilled liquids. Add new desiccant, as instructed in subsection C, above.

i. Go to steps 4 - 8, below.

4. If the lamp was removed, reinstall it. See Appendix 8.

5. If the Emission Filter Holder was removed, install Emission Filter Holder, and (**Important**) tighten hex screw on the end of the Holder to restore o-ring moisture-seal. (See Appendix 8.)

6. Put the Sample Compartment cover in place (align Excitation Filter Holder carefully), and install all 8 screws very loosely.

   If the Excitation Filter Holder was removed from the cover, install the Excitation Filter Holder and check alignment of Sample Compartment cover and Holder. Holder should be fully inserted with no obvious space at insertion point.

   Progressively tighten all Sample Compartment screws until they are snug, but not dead tight.

   Important: Tighten hex screw on the end of the Excitation Holder to restore o-ring moisture-seal. (See Appendix 8.)

7. If you have changed cuvette size or to a new application, you may have to readjust basic operating level using the Sensitivity Adjustment Knob. Try calibrating your instrument first following your normal procedure. (See Appendix 6B.)

8. Calibrate the fluorometer. (See Section 3F.)

G. Water in the Sample Compartment

If water does enter the Sample Compartment:

1. Turn power off. There is no hazard, since the lamp compartment is separated from the sample compartment, but readings will be affected.

2. Remove the Filter Holders. (See Appendix 8.)

3. Remove the Sample Compartment Cover (8 hex screws). Remove the lamp. See Figure A2 and Appendix 8.

4. If the water that entered the Sample Compartment was salt water, flush the affected area with fresh water, then with deionized or distilled water.

   If only fresh water entered the Sample Compartment, flush the affected area with deionized or distilled water.

5. Check the filters for moisture or damage. Clean, dry, or replace as necessary.

6. Make sure PM tube window and lamp window are clean and free of debris (use only distilled water and a soft cloth to clean). Dry completely inside Sample Compartment with gentle heat. (A hair dryer works.)

7. Reassemble and return to service.

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Installation of the 10-AU-020 or 10-AU-080 25mm One-Piece Flow Cell

1. Remove the discrete sample adapter or three piece 25mm flow cell as specified in Section E of Appendix A-7 of the 10-AU User's Manual.

2. At this point, the cover should be off the lamp housing, and the excitation filter and lamp should be removed.

3. Screw in the gray valve with the red handle into the bottom of the flow cell so that it is hand tight. The assembly should look like the flow cell depicted in Figure 1. The distance between the flow cell body and the mounting plate should be about 2 1/8 inches.

4. Remove the three hex head screws from the handle mount on the flow assembly with the tool that is provided with your 10-AU fluorometer. It is kept on the 10-AU to the left of the power and signal cable connection. Remove the left half of the mounting plate.

5. Lube the two o-rings on the flow assembly with the o-ring grease provided. Insert the one-piece assembly through the hole at the bottom of the sample compartment, and slide it in half way. (See Figure 1)

6. If you have temperature compensation, attach the gray temperature compensation connector wire to the connector located underneath the sample compartment.

7. Insert the one-piece assembly the rest of the way in until it can go no further.

8. Attach the handle mount you removed in step 3 to the left handle of the 10-AU by installing the three hex head screws with the hex head tool. (See Figure 2)

9. Tighten the set screws in the sample compartment that will secure the one-piece flow cell in place.

10. Reinstall the lamp and the excitation filter that you removed in step 1 according to the instructions in Appendix A-7.

11. Install the cover plate on the lamp housing.

12. Install the silver colored pipe adapter to the top of the flow cell assembly. (See Figure 2)

13. Attach your pipe or garden hose attachments to the fluorometer. Remember: the water should flow from bottom to top on the fluorometer (i.e., the water inflow should attach to the bottom).

14. Make sure that once you are ready to flow water through the fluorometer that the red handle on the gray valve is parallel to the flow path (horizontal). If it is vertical, the flow is stopped by the valve.

15. When calibrating, turn the red handle on the valve to the vertical position and inject a standard with the syringe. You will probably have to inject two or three times to clear the previous sample entirely out of the cell. When you are done calibrating, turn the red handle to the horizontal position to resume the flow.

16. To clean the flow cell, first stop the flow of water by turning the red handle on the valve to the vertical position. Unscrew the large silver colored plug on the top of the flow assembly with a flat head screwdriver. Use a test tube brush to clear any algae or other material that may be "fouling" the cell. When you are done scrubbing the inside of the flow cell, reinstall the plug, and turn the red valve handle to the horizontal position.

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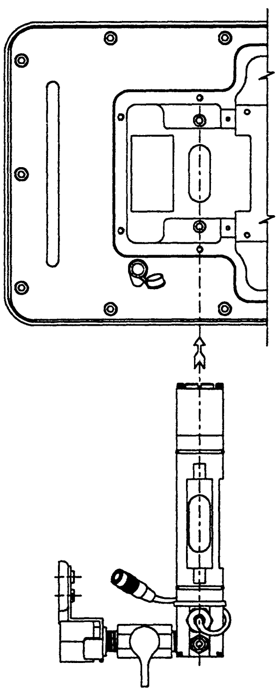
Note of Caution:
Components of the flow cell (Continuous-Flow Cuvette System) are made of PVC, Delrin, and/or nickel-plated brass; and the seals are made of elastomers suitable for use in fresh water and marine environments. When using the Continuous-Flow Cuvette System, DO NOT use organic solvents such as acetone, methanol, or pyridine, or corrosive materials such as strong acids and bases.

One-Piece Flow Cell Drawings



|2 1/8 |
Figure 1

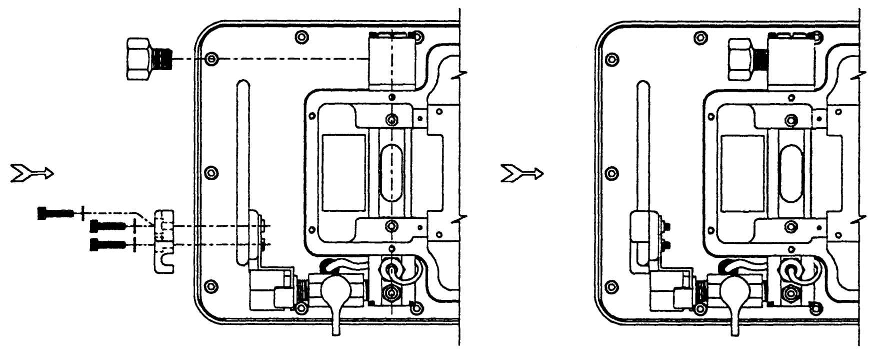


Figure 2

Appendix 8
FILTER AND LIGHT SOURCE REPLACEMENT

Readings on a given fluorometer are affected by the unique properties of the individual lamp and filters installed. Therefore, please note the cautionary remarks about lamps and filters when replacing them.

NOTE: If you change to a different cuvette size or a new lamp or filters, you will have to readjust the Sensitivity Adjustment Knob. DO NOT ADJUST the Sensitivity Adjustment Knob otherwise: It is not necessary and it will disrupt your study. (See Appendix 6B.)

Filters. There are three different types of filters on your fluorometer (all located inside the Sample Compartment): the reference filter, the excitation filter (mounted in a holder that fits around cuvette), and an emission filter(s). See Figure A2 for their locations. They are made of glass and will break if dropped, so handle them carefully.

Although filter problems are not common, missing, broken, or improperly mounted filters can result in erratic or unstable readings, high or low readings, or the inability to blank to zero. See Troubleshooting, Section 4B. If you are having problems that might be caused by filters, verify that you have the correct filters for your application, and that they are properly installed. (See Appendix 9 for a description of the filters needed for your application.)

Many filters are laminated (a plastic filter sandwiched between two layers of glass). With age and exposure to moisture, a filter can separate; i.e., it will no longer have a uniform color and density. To check the condition of a filter, remove it and hold it up to a bright light. Color and density should be fairly consistent; some wrinkling is acceptable. It should be replaced if it is badly separated, especially in the center portion. In the case of a mirrored filter, it should be replaced if it is flaking or freckling (look closely - freckling can be minute).

Lamp. You will be notified of a bad lamp by the "Lamp is Off/Bad" alarm. If this alarm is triggered, open the Sample Compartment and check the lamp through the viewport. But, note that it is possible for the lamp to emit a weak light, appearing to be on, and still need replacing. The lamp should be a uniform solid color over the entire length. If it is not, make sure it is properly seated on both ends. Replace the lamp if it is flickering, or only lit at one end.

⚠ **Ultraviolet Light.** In some applications such as Rhodamine WT and Short Wavelength Oil, the light source is supplied by a clear quartz lamp, which is a source of ultraviolet light. This light can cause permanent damage to the eyes if observed. The lamp is contained in the sample compartment, which prevents any hazard during normal operation. When the sample compartment cover is removed for any reason, a safety interlock switch turns the lamp off. **DO NOT** override this safety switch. You must wear approved protective goggles whenever there is a potential for exposure to ultraviolet light from the quartz lamp. The instrument should be turned off and unplugged before removing or changing the lamp.

Replacing the Filters and Lamp

The 10-AU-005-CE Fluorometer is equipped with the rapid-change excitation and emission filter system. Refer to Figure 1 for the location of the filter holders. See Appendix 9 to see what filters and lamp are required for your application.

Procedure:

1. Turn off the power.

2. Filter Holders are designed with an o-ring seal against moisture in the sample compartment. The seal must be loosened before Filter Holders can be removed. Use the 5/32" Allen wrench supplied to loosen the hex nut on the end of each Filter Holder; this will break the seal and allow Holders to be removed. (See Figure A2.)

3. Grasp the Excitation Filter Holder (front) and slide it out of the instrument toward you.

   The round one inch diameter filter will be held in place by a metal retainer ring or by a small hex screw (for very thick filters).

   Examine the filter's condition by holding it to a bright light. Set it aside.

4. Grasp the Emission Filter Holder (to the left of the Sample Compartment) and slide it to your left and out of the instrument.

   The round one-inch diameter filter will be held in place by a metal retainer ring or by a small hex screw (for very thick filters).

   Examine the filter's condition by holding it to a bright light. Set it aside.

5. To install new filters already mounted in holders, simply slide the Excitation Filter Holder with the new filter and the Emission Filter Holder with the new filter into the proper slots in the Sample Compartment. The holders are labeled and designed to fit only in the proper slots in the correct orientation. We recommend that you check to see that the filter itself is correct and installed in the right holder, properly oriented before proceeding.

   If you are changing applications or replacing damaged filters, see Appendix 9 to determine the proper filter(s).

6. To replace the excitation filter in a holder:

   a. If the filter is held in place by a hex screw (no metal retainer ring), then use a 0.05" Allen wrench to loosen the hex screw. Place the holder back side up (with the "lip" side of the filter opening up) and push the filter out by pressing evenly on the edges of the filter with two fingers. Remember that the filter is glass and can be broken or scratched.

      Locate the correct filter (Appendix 9).

      NOTE: If an attenuator plate is necessary, install it by placing it on top of the excitation filter (closest to the lamp) before installing the retainer ring or tightening the hex nut.

      If installing an interference filter (mirror-like filter set in a black ring), it must be installed with the mirrored side toward the lamp. Place the holder on a flat surface with "lip" side of the opening down. Put the filter in, mirrored-side up. Tighten the hex nut. DO NOT overtighten.

      It does not matter which side of the colored glass filters faces the lamp. Place the filter in the holder opening and tighten the hex nut. DO NOT overtighten.

      Wipe off any fingerprints from the filter with a lint-free wipe.

   b. If the filter is held in place by a metal retainer ring, then place the holder on a flat surface, ring side down, and push the filter out by pressing evenly on the edges of the filter with two fingers. If the filter doesn't come out with gentle pressure, try removing the metal ring first (it slides out the front of the holder). Remember that the filter is glass and can be broken or scratched.

      Locate the correct filter (Appendix 9).

      NOTE: If an attenuator plate is necessary, install it by placing it on top of the excitation filter (closest to the lamp) before installing the retainer ring or tightening the hex nut.

      If installing an interference filter (mirror-like filter set in a black ring), it must be installed with the mirrored side toward the lamp. Place the holder on a flat surface with the "lip" side of the opening down. Put the filter in, mirrored-side up. Put the retainer ring on top and work it evenly into the opening until it is flat and snug against the filter.

It does not matter which side of the colored glass filters faces the lamp. Place the filter in the holder opening; put the retainer ring on top and work it evenly into the opening until it is flat and snug against the filter.

Wipe off any fingerprints from the filter with a lint-free wipe.

7. To replace the emission filter(s) in a holder:

   a. <u>If the filter is held in place by a hex screw</u> (no metal retainer ring), then use a 0.05" Allen wrench to loosen the hex screw. Then, place the holder with the "lip" side of the holder opening up, and push the filter out by pressing evenly on the edges of the filter with two fingers. Remember that the filter is glass and can be broken or scratched.

      Locate the correct filter(s). See Appendix 9.

      If using one of the interference filters (mirror-like filter set in a black ring), it must be installed with the mirrored side toward the sample. Place the holder on a flat surface with the "lip" side of the opening down. Put the filter in, mirrored-side up. Tighten the hex nut. DO NOT overtighten.

      The colored glass filters must also be installed in the right direction. If the filter is a combination filter, it will have an arrow on the side; the filter will be installed so the arrow points away from the sample. Place the holder on a flat surface with the "lip" side of the holder opening down. Put the filter in so the arrow points down, toward the back side of the holder. If more than one filter is to be installed in combination, then place the filter that is to go nearest the photomultiplier in first, and the filter that is to go nearest the sample in last. Finally, tighten the hex nut. DO NOT overtighten.

      Wipe off any fingerprints from the filters with a lint-free wipe.

   b. <u>If the filter is held in place by a metal retainer ring</u>, then place the holder on a flat surface, ring side down, and push the filter out by pressing evenly on the edges of the filter with two fingers. If the filter doesn't come out with gentle pressure, try removing the metal ring first (it slides out the front of the holder). Remember that the filter is glass and can be broken or scratched.

      Locate the correct filter (Appendix 9).

      If using one of the interference filters (mirror-like filter set in a black ring), it must be installed with the mirrored side toward the sample. Place the holder on a flat surface with the "lip" side of the holder opening down. Put the filter in, mirrored-side up. Put the retainer ring on top and work it evenly into the opening until it is flat and snug against the filter.

The colored glass filters must also be installed in the right direction. If the filter is a combination filter, it will have an arrow on the side; the filter will be installed so the arrow points away from the sample. Place the holder on a flat surface with the "lip" side of the holder opening down. Put the filter in so the arrow points down, toward the back side of the holder. If more than one filter is to be installed in combination, then place the filter that is go nearest the photomultiplier in first, and the filter that is to go nearest the sample in last. Finally, put the retainer ring on top and work it evenly into the opening until it is flat and snug against the filter.

Wipe off any fingerprints from the filters with a lint-free wipe.

8. If you have changed applications, you may have to change the lamp. Locate the Lamp for your application. (See Appendix 9.)

Ultraviolet Light. In some applications such as Rhodamine WT and Short Wavelength Oil, the light source is supplied by a clear quartz lamp, which is a source of ultraviolet light. This light can cause permanent damage to the eyes if observed. The lamp is contained in the sample compartment, which prevents any hazard during normal operation. When the sample compartment cover is removed for any reason, a safety interlock switch turns the lamp off. **DO NOT** override this safety switch. You must wear approved protective goggles whenever there is a potential for exposure to ultraviolet light from the quartz lamp. The instrument should be turned off and unplugged before removing or changing the lamp.

9. Remove the Sample Compartment cover by unscrewing the hex nuts retaining the cover.

10. Remove the lamp by rotating it 90 degrees and pulling it out toward you. **BE CAREFUL; IT MAY BE HOT.**

    NOTE: <u>If you are changing filters within the same application and desire to maintain calibration, you should mark the Lamp so that it may be returned to its original position.</u>

11. Take the new lamp and install it by inserting the two end prongs into the slots on the upper and lower lamp sockets and turning 90 degrees.

    If you are reinstalling the old lamp and wish to maintain calibration, the lamp should be reinstalled in the original position. See paragraph 10.

12. If you are changing applications, you may have to change the reference filter. Locate the Reference Filter for your application. Refer to the Filter Selection Guide, Appendix 9, for details.

    The reference filter is mounted to the right of the lamp, behind two metal spring clips. Remove the old filter by sliding it toward you from behind the two clips. Check its condition by holding it to the light.

    To install the reference filter, slide it behind the two spring clips (Figure A2).

13. Replace Emission Filter Holder, and (**Important!**) tighten hex screw on the end of the Holder to restore o-ring moisture-seal. (See Appendix 8.)

14. Put the Sample Compartment cover in place and install all 8 screws loosely.

    Replace the Excitation Filter Holder and check alignment of Sample Compartment cover and Holder. Holder should be fully inserted with no obvious space at insertion point.

    Progressively tighten all Sample Compartment screws until they are snug, but not dead tight.

    Important: Tighten hex screw on the end of the Excitation Holder to restore o-ring moisture-seal. (See Appendix 8.)

15. If you have changed cuvette size or to a new application, you may have to readjust basic operating level using the Sensitivity Adjustment Knob. Try calibrating your instrument first following your normal procedure.

16. Recalibrate your fluorometer. (See Section 3F.)

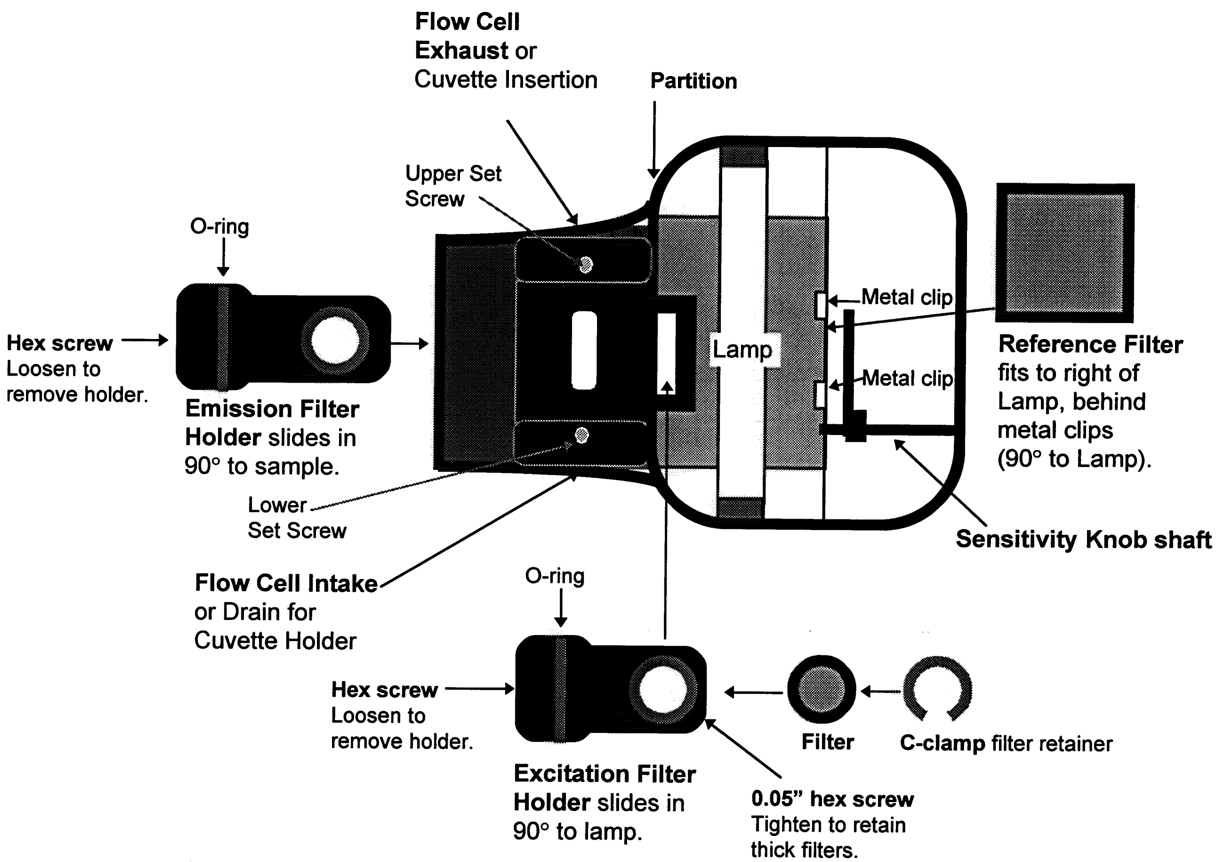


Figure A2. Sample Compartment (cover removed)

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Appendix 9
FILTER SELECTION

A. Theory of Selection

Fluorometric analysis is based on the measurement of fluorescent materials absorbing light at one wavelength and converting it into light at a longer wavelength.

Two primary considerations in selecting the proper filter, light source, and light detector are:

1. The light source and excitation filter must allow light (which the material being analyzed absorbs at certain wavelengths) to fall on the sample.

2. The light detector and emission filter system must be sensitive to the wavelength of light emitted by the material being analyzed.

The limit of sensitivity of the Model 10-AU fluorometer is almost always determined by the level of extraneous background light that reaches the light detector. This background light may vary from sample to sample.

Four primary sources of such unwanted light and their impact on filter, light source, and light detector selection are:

1. Interference from other fluorescent material(s) in the sample.

   By proper choice of the wavelength of light that falls on the sample and wavelength to which the light detector is sensitive, system sensitivity to the fluorescent material being analyzed can be maximized, while sensitivity to interfering fluorescent materials is minimized.

2. Scattered light in the system from either reflective surfaces or from turbidity.

   This becomes a problem when certain inherently fluorescent filters are used. These filters convert scattered light from one wavelength to another. If the final wavelength is also transmitted by the filter, it acts as an extraneous light component and will reach the light detector.

   This fluorescence of the emission filter becomes more of a problem when measuring low concentrations with high and variable turbidity. If the turbidity is constant, then it simply constitutes blank, on the assumption that it was present identically in blank, standard, and samples.

Intermediate blocking filters can reduce this interference by protecting inherently fluorescent filters from scattered light. The order of placement of these filters is important.

3. Overlap in the transmission wavelength ranges of excitation and emission filters.

   If both the excitation and emission filters transmit even a small percentage of the same wavelength of light, excitation light scattered by fixed components of the sample system and turbidity in the sample can also reach the light detector. Such "overlap" should be held to a minimum.

4. Raman fluorescence of the solvent.

   This interference, which is often significant, can be minimized by proper selection of filters that prevent the Raman fluorescence from reaching the light detector.

   Raman will not vary from sample to sample. It will contribute to an increased blank, which does add noise when reading low concentrations.

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OPTICAL FILTERS

Turner Designs fluorometers require an excitation and an emission filter; the Model 10-AU also uses a reference filter. Optical filters are chosen to be optimal for each application, cost effective, and durable. Filters are used to selectively pass a portion of the ultraviolet or visible spectrum.

In combination with a light source, the excitation filter allows only light which excites the molecule of interest to strike the sample. The emission filter allows the fluorescence from the sample to pass to the detector and blocks stray light from the light source or interfering components in the sample. The reference filter is used in the reference path of the 10-AU series and is a factor in determining the basic operating level of the instrument.

Filters can be used alone or in combination to select the desired spectral band. Optical filters obey the Bouguer-Lambert Law, which states that the spectral transmittance of two or more optical filters used simultaneously is equal to the product of the spectral transmittance of each filter.[1]

Filters with four types of spectral characteristics are used in Turner Designs fluorometers: broadband, narrowband, sharpcut, and neutral density.

- A **broadband** filter can pass a broad band of light. For instance, a broadband filter may transmit light from 300 - 400 nm, but block light with wavelengths shorter than 300 and longer than 400.
- **Narrowband** filters pass a narrow band of light (as little as 1 nm). For example, a 436 nm filter with a bandpass of 10 nm, will pass light from 431 - 441 nm (5 nm on either side of 436 nm).
- **Sharpcut** or edge filters can be used to block light that is longer or shorter than a nominal wavelength. A 450 nm long-wave filter will allow transmission of light that is longer than 450 nm, but it will block light that is shorter than 450 nm. A 450 nm short-wave filter will transmit light that is shorter than 450 nm and block light that is longer than 450 nm.
- A **neutral density** filter, primarily used as a reference filter, can be used to decrease the transmitted light across a very broad spectrum. For instance, a neutral density filter can be used to decrease the total light transmission by a factor of 10 or 100.

Three types of optical filters are used in Turner Designs Fluorometers: 1. Optical Glass, 2. Interference, and 3. Gel Wratten.

1. ***Optical Glass Filters.*** Optical glass filters are made from glass that absorbs specific wavelengths of the spectrum. They are relatively inexpensive and are very durable under most conditions. Both bandpass, sharpcut, and neutral density filters are available in optical glass. However, the choice of filter glasses is limited. The amount of transmission and band width is dependent on the glass thickness. The following factors may affect optical glass:

   - Thermal shock caused by a rapid temperature change;
   - Solarization caused by prolonged exposure to ultraviolet light can cause an increase in absorption (decrease in transmission);
   - Exposure to high humidity or corrosive environments can cause 'spotting' or 'staining', which changes the surface, resulting in increased light scattering off the surface and decreased transmission through the glass.[2]

   However, we have found that glass filters can be used for years or decades under most conditions.

2. ***Interference Filters.*** In terms of spectral characteristics, interference filters can have broad or narrow bandpasses, or can be sharpcut filters. Interference filters used in Turner Designs fluorometers are primarily narrow bandpass. Interference filters are made by coating optical glass with two thin films of reflecting material separated by an even-order spacer layer. The central wavelength and bandwidth of the filter can be controlled by varying the thickness of the spacer layer and/or the number of reflecting layers. To ensure out-of-band blocking — blocking undesirable wavelengths of light — an additional blocking component is added. While the additional blocking eliminates out-of-band light transmission and decreases background noise, it also decreases the overall light transmission through the filter which decreases the fluorescent signal. Interference filters typically permit 10 to 70% light transmission. The minimum specified transmission depends on the transmitted wavelength and bandwidth.

   Interference filters are affected by temperature. The center wavelength will shift linearly with, and in the direction of, changes in temperature. For example, the temperature coefficient for a 400 nm filter is about 0.015 nm/°C. The center wavelength and maximum transmission of interference filters can drift with age, especially under conditions of high humidity and variable temperatures.[3] Good quality filters are hermetically-sealed to mitigate the affects of aging. Hermetically sealed filters are guaranteed for one year; we have found that under good ambient conditions, such as in a laboratory, the filters show minimum signs of aging after two years or more.

   A new interference filter usually has a uniformly dark side and a uniformly reflective or mirrored side. To protect the filter from heat and light, the reflective side should always face the light source. A filter that is affected by age and humidity will show discoloration around the outside diameter, this discoloration will move toward the center of the filter with time and additional damage. A symptom of aging is a significantly decreased maximum transmission which results in less sensitivity for a fluorescent assay. The recommended operating conditions for interference filters is -40°C to +70°C, and a maximum temperature change of 5°C/minute.[3]

3. ***Gel Wratten Filters.*** Gel Wratten filters can have broad or narrow bandpasses or can be sharpcut filters. Gelatin filters are made by dissolving specific organic dyes into liquid gelatin. The gelatin is coated onto prepared glass and when it is dry, it is stripped off the glass and coated with lacquer. Each filter is standardized for spectral transmittance and total transmittance. At Turner Designs, the gelatin filter is placed between two pieces of glass or in combination with other filters for use in the fluorometer. Like dyes in other applications, the spectral characteristics of the dyes used in filters may change depending on the dye used, age, and exposure to heat and light. Gelatin filters should be kept cool, dry and should not be subjected to temperatures higher than 50°C.[1] Most of the gelatin filters used by Turner Designs have been found to be stable under test conditions, which include up to two weeks of continuous exposure to several light sources.

References

[1] Kodak Filters for Scientific and Technical Uses, Eastman Kodak Company, 3 ed. 1981.
[2] Schott Color Filter Glass, Schott Optical Glass Inc., 1976
[3] Andover Corporation Optical Filter Guide, Andover Corporation.

Appendix 10
MODEL 10-AU FLUOROMETER SENSITIVITY

Note that the sensitivity limits set forth below are **estimations only**. In most cases, using a different cuvette size in combination with selected filters will permit measurements of concentrations satisfactory for your study. Contact Turner Designs to discuss special cases.

A. Oil Measurements.

The aromatic hydrocarbons in petroleum and petroleum by-products are naturally fluorescent. See Appendix 3 for more information on types of oil studies.

<u>25 x 150 mm Cuvette Holder or Continuous-flow Cuvette</u>. Using the short wavelength oil optical kit, with no sample treatment, concentrations of crude or very heavy oils have been detected on a continuous-flow basis in sea water as low as 5 parts per billion (ppb). Extractive techniques allow detection at even lower concentrations: detectability at 0.2 ppb has been reported. Using the long wavelength optical kit, the limit of detectability is about 0.1 ppm; the linear range extends to about 50 ppm; and the useful range using a calibration curve extends to about 200 ppm.

Concentrations of #2 fuel or lighter oils have been detected using the short wavelength oil filters at about 2 ppb; the linear range extends to about 2 ppm; and the useful range using a calibration curve is about 10 ppm.

Recently, we have measured gasoline and benzene as low as 15 ppb, and are currently studying ways to further increase sensitivity.

<u>13 x 100 mm Cuvette Holder or Continuous-flow Cuvette</u>. Sensitivity is approximately 20% of that of the 25 mm. The shorter path length allows higher concentrations to be read, with an increase in the linear range.

<u>3 mm Continuous-flow Cuvette</u>. This flow cell allows measurements at even higher concentrations than the intermediate size. The shorter path length increases the linear range and reduces the effects of light-absorbing materials. We have read processing and cutting oils linear to 1000 ppm, and using a calibration curve in the parts per thousand range.

<u>1 mm Continuous-flow Cuvette</u>. We have measured crude oil linear to 250 ppm, with the useful range using a calibration curve as high as 1000 ppm.

B. Chlorophyll and Pheophytin.

All chlorophyll-containing organisms are fluorescent. Where the organisms are small, such as phytoplankton, fluorescence may be measured directly without extraction or chemical treatment. Sensitivity of the fluorometer varies, depending upon such factors as the amount of organic substance associated with a given quantity of plant pigment, the presence of humic materials, and the fluorescence efficiency of the particular species. However, the fluorometer is at least 20 times more sensitive than spectrophotometric techniques.

Continuous-flow Cuvette. *In vivo* measurements are commonly used at open-ocean levels, with the either the 13 mm or 25 mm flow cell. The fluorescence of chlorophyll in intact cells exhibits about one-tenth (0.1) the intensity of acetone extracts (Lorenzen, Carl J., "A Method for the Continuous Measurement of *In Vivo* Chlorophyll Concentration," *Deep-Sea Research* 13:223-227 (1966).

Cuvette Holder. The 13 mm Cuvette Holder is normally used with extractive techniques. The limit of detectability is about 25 parts per trillion in the final extract. With the 25 mm Cuvette Holder, sensitivity would be increased about 5-fold (5 parts per trillion).

The US Fnvironmental Protection Agency has authored a new standard method for extracted chlorophyll. Method 445.0 found detection limits of .05 µg/L (ppb; or 50 parts per trillion); the upper limit of linearity was 250 µg/L. Contact Turner Designs for Method 445.0, "*In Vitro* Determination of Chlorophyll *a* and Pheophytin *a* in Marine and Freshwater Phytoplankton by Fluorescence."

For information about linearity and factors affecting measurements, ask for our monograph "Chlorophyll and Pheophytin."

3 mm Continuous-flow Cuvette. Allows measurements at higher concentrations than the intermediate size. The shorter path length increases the linear range and reduces the effects of light-absorbing materials. (Contact Turner Designs for details.)

C. Fluorescent Tracer Studies.

25 x 150 mm Cuvette or Flow Cell. The limit of detectability in pure water of the most commonly used fluorescent dyes (Rhodamine WT, Rhodamine B, and fluorescein) is about 10 parts per trillion. Pontacyl brilliant pink is less detectable by a factor of three. The linear range extends to about 0.1 ppm (active ingredient); and the useful range using a calibration curve is 0.5 ppm. Factors such as fluorescent background affect the detectability. In raw sewage, however, measurements can be made to 0.1 ppb of Rhodamine WT. Refer to Appendix 3 for more information on fluorescent tracer studies.

Appendix 11
DATA COLLECTION

A. External Data Logging

The Model 10-AU-005-CE is configured to output both voltage (analog) and serial signals to external data collection devices. The voltage output parameters appear on screen 1.4, and the serial data output parameters appear on screen 1.5. They are <u>displayed</u> on the Model 10-AU-005-CE even if you are not connected to an external device. They are <u>operational</u> only in conjunction with an external data collection device connected to the 10-AU through the AC or DC Power & Signal Cable.

The voltage and serial signals can be used simultaneously. For example, voltage output could be sent to a chart recorder at the same time serial output was being sent to a computer.

Refer to Table 6 for default values and ranges for these parameters.

To log data with the 10-AU using Windows®, contact Turner Designs for information about the 10-AU-005-CE Fluorometer Spreadsheet Interface Software (P/N 10-AU-4400). (See Appendix 11, section B.)

1. <u>Screen 1.4: Output (Voltage Output)</u>

   1.41 Full scale voltage
   1.42 Zero point
   1.43 Full scale

   To access screen 1.4, from the Main Menu, press <1>, Operational Parameters, then press <4>.

   The voltage output can be sent to a chart recorder or data logger. The output is proportional to the reading on the HOME screen; it is continuous and updated once every second.

   NOTE: If the Model 10-AU is in the auto-range mode, when it changes ranges the readout on the HOME screen and the voltage output will "freeze" for 5 seconds. During this 5 seconds, this "frozen" output is what will be sent to a chart recorder or data logger.

   a. <u>Full scale voltage</u>. The user can set 0.1, 1, 2, or 5 V as full scale. This allows flexibility in connecting to different data loggers or chart recorders.

b. Zero point. The user can select what sample concentration will correspond to the output zero point and the output full scale. For example, the user could assign 100 ppm to zero and 900 ppm to full scale. The narrower the range, the greater the resolution. However, if, for example, zero volts is set to correspond to 40 and 5 volts (full scale) to 60, then any reading below 40 would be output as zero, and any data above 60 would be output as greater than 5, with a maximum of 5.12 volts.

c. Full scale. The user can select what sample concentration will correspond to the output full scale. See the example in paragraph b.

2. Screen 1.5: Serial Data Out

1.51 Baud rate
1.52 Set interval
1.53 Set index
1.54 Set format*

* visible only if the optional temperature-correction package has been purchased and the temperature probe is plugged into the instrument

The serial output signal can be sent to a computer or serial logger or printer. Serial data is sent out in seven significant figures (XXXX.XXX). The data sent out are the sample readings from the HOME screen (except readings will be in seven, rather than three, significant figures).

When used in conjunction with a computer, serial data is sent out in real-time, on the selected interval, and displayed on the computer with the index number, the date, time, and concentration readout or relative fluorescence.

NOTE: If the 10-AU is in the auto-range mode, when it changes ranges, the readout on the HOME screen will "freeze" for 5 seconds. During this 5-second period, the "frozen" reading is the one that will be sent to any data collection device.

Detailed instructions for using the 10-AU in conjunction with a computer are found in Appendix 11B.

To access screen 1.5, from the Main Menu, press <1>, Operational Parameters, then press <5>.

a. Baud rate. Allows the user to select either 9600 or 4800 baud rate, as compatible with your computer.

b. Set interval. The user can determine how often data will be sent out to a computer or logger, from 0 to 3600 seconds.

   There is an additional feature, a Manual Serial Data Output, which allows you to output a reading to the computer any time, simply by pressing 1 - 8 on the keypad, while on the HOME screen. For example, if you have selected a 10-second interval, the 10-AU will output a reading to the computer every 10 seconds. From the HOME screen, if you press <2> during the 10-second interval, on the keypress, a reading will be sent to the computer with the marker "2" displayed in front of the index number for that reading.

c. Set index. The user may select a number from 0 - 9999 to begin numbering a set of data. For example, if you select an index of 100, with an interval of 10 seconds, and begin logging, after 10 seconds the first reading will be sent to the computer with an index number of 0100; in another 10 seconds the second reading will be sent, numbered 0101, etc. When 9999 is reached, the index will return to 0.

d. Set format. The user may decide to send out "data only" or "data plus temperature" to a computer or data logger. (This function will appear only if you have purchased the optional temperature-compensation package and the temperature probe is plugged into the instrument.)

Table 6. External Data Logging Defaults and Ranges

Data Logging Parameter	Default	Range
Output full scale voltage	2V	0.1, 1, 2, 5 V
Output zero point	0	0 - 9998
Output full scale	999	1 - 9999
RS-232 baud rate	9600	9600/4800
Serial data out interval	5 (SEC)	0 - 3600 (SEC)
Serial data out index	0	0 - 9999
Serial data out format*	Data only	Data only/data+temp

* Visible only if equipped with the optional temperature-correction package, and temperature probe is plugged in

3. Serial Data Output Information for Programmers.

(See Appendix 11H for Power/Telemetry Connector pin inputs and outputs.)

If you plan to interface your 10-AU with other instruments and need to write a program to do so, a definition of the serial data output string follows. If you are using the Turner Designs Data Collection Software (see Appendix 10B), you do not need to review this subsection.

Serial Data Protocol: RS-232 asynchronous; 4800/9600 baud; 8 data bits; 1 stop bit; no parity check.

Here are three sample lines of data sent out from the fluorometer (100% ASCII):

```
0  0013 ::  4/23/92 21:34:25 = 55.429 (mg/kg)   83.4 (F)
0  0014 :: 10/23/92 11:02:07 = 10.009  (PPM)    27.8 (C)
0  0015 ::  4/23/92 09:18:31 = 40.223 (mg/l)    44.4 (F)
 ⇑⇑     ⇑  ⇑        ⇑        ⇑ ⇑      ⇑      ⇑⇑      ⇑
                                    or⇑⇑    or⇑⇑⇑
```

Each line starts with ASCII 0X0D (carrier return) and ASCII 0X0A (linefeed), and:

0	= Marker (0 to 8)
0013	= 4-digit index (0000 to 9999)
::	= Separator (2 ASCII 0X3A)
4/23/92	= Month/Date/Year
21:34:25	= Hour:Min:Sec
=	= Separator (ASCII 0X3D)
55.429	= Signal (Maximum 7 digits: XXXX.XXX)
(mg/kg)	= Signal units of measurement
83.4	= Temperature (to one decimal)*
(F)	= Temperature nomenclature*
(blank)	= No terminator
	* if equipped with temperature compensation
⇑	= Arrows indicate the number of spaces between data items.

There may be one or two spaces (indicated by ⇑) after the signal, depending on the signal units of measurement you choose. If you choose (RAW) or (PPM) or another three-character unit, then there will be two spaces. If you choose units with more than three characters (for example, mg/l), then there will only be one space. After the units, there may be two or three spaces, depending on the units chosen. If you choose units with five characters (i.e., mg/kg), then there will be two spaces. For three or four character units (i.e., PPB or mg/l) there will be three spaces.

Example. If you wanted program your computer so just the signal, the units, and the temperature were captured, you would look for the equal sign. One space after the equal sign the signal begins; it ends with a space. After the space (or spaces), look for a parenthesis "("; the units of measurement begin with "(" and end with ")". After the ")", look for two or three spaces, then the temperature readout begins; temperature ends with a space. The temperature nomenclature (F or C) is then found between the last "(" and ")".

B. Spreadsheet Interface Software (used with external data logging)

The Spreadsheet Interface Software (P/N 10-AU-4400) was written by Turner Designs to allow discrete sampling data or continuous flow cell data from the fluorometer to be sent directly to an Excel® spreadsheet. The data can either be sent manually to the spreadsheet or at a programmed rate ranging from 0-3600 seconds (see section A2 of Appendix 11). Please contact Turner Designs for more information.

Note: If you are using a DOS-based system, contact Turner Designs about the Data Collection Software (DCS) program, a DOS-based system written specifically to interface with the fluorometer's external data logging capability. Using DCS, data will be sent in real-time directly to an external computer (IBM-compatible). Data includes a marker, index, date, time, sample readout (to seven significant digits), and temperature if your fluorometer is equipped with temperature compensation. The amount of data you can log is limited only by the disk memory of your computer. DCS saves data in ASCII format for manipulation with standard computer programs.

C. Serial Data Collection with a Macintosh Computer

The serial data output function on Turner Designs' instruments is fully compatible with a Macintosh computer. Turner Designs instruments send out an ASCII signal, which is readable by most standard Macintosh-compatible programs.

You will need the appropriate cables and adapter to establish the connection. If you do not already have the required cables, Turner Designs offers a Macintosh Cable Kit (P/N 7000-940).

Refer to Turner Designs operation manual for instructions about data output settings on instrument.

Data Capture Using a Macintosh Desktop Computer

1. Connect your computer to the RS-232 port on the instrument with the cables included in the kit. (computer ⇒ round 8 pin-25 pin cable ⇒ 25 pin/9 pin adapter ⇒ RS-232 cable (came with instrument) ⇒ instrument).

2. Access **File** (from toolbar) ⇒ Create a **New Folder** in which the data can be stored.

3. On your computer, open the communications program you have chosen to use (Z modem, Z-term, Microphone II, Clarisworks, any terminal program, etc.). For details on operating your communications program, refer to program manual.

   NOTE: If there is no appropriate communications software on your computer you can download Z-modem from most shareware sites on the Internet.

   If all communication settings are already set up on the instrument and computer, data will appear on the screen and you can skip steps 4 and 5.

4. Depending and which model you have, either; access **apple menu** ⇒ **control panels** ⇒ **communications** **(or)** access **settings**(from toolbar) ⇒ **connection.**

5. Once in **communications/connection**, set **baud rate** = 9600, **data bits** = 8, **stop bits** = 1.

6. When you are ready to begin measurements, access **File** (from toolbar) ⇒ **Start Capture,** when asked where to send data, choose the **new folder** you created in Step 1.

7. Set your fluorometer for real time serial data logging. If you want to log data continuously, select an interval from 1 - 3600 seconds on screen 1.52. If you want to log discrete data, set the interval to 0 on screen 1.52. Then, when you want to log a data point, press a number <1> through <8> on the fluorometer keypad. Data should appear on computer screen.

   Example: To log discrete sample readings, press any number <1> through <8> from the HOME screen. A row of data will be sent to your computer, e.g.:

   ```
   1 1242 :: 6/07/93 17:03:57 = 25.789 (PPM)  26.2 (C)
   ```

   Each time you wish to log a sample reading, press a number <1> through <8> on the fluorometer keypad.

8. When data transfer is complete, access File (from toolbar) and select **Stop Capture**. The active window will continue to display downloading data. To exit, access **File** ⇒ choose **Quit**. To view data, access the folder that was made in Step 1. From this folder the data can be moved to a spreadsheet program through the **transfer** function or through **cut and paste** functions.

Data Capture Using a Macintosh Laptop Computer

Because laptop computers have only one serial port, establishing a connection with a Turner Designs instrument can be a more complex process compared to use of a desktop. However, once the connection has been established and setup noted, the process is identical to the desktop procedure.

When starting computer, hold down the **Shift** key to turn off all extensions. This will free up any programs that could be occupying the serial port. However, some of the extensions may be needed for data transfer and therefore each laptop user must go through a process of trial and error to find the essential extensions. (**NOTE:** Every computer varies in extensions and therefore must be handled on a case by case basis)

Once the computer is booted up with extensions off, follow desktop instructions 1-5.

If no data appears in window:

1. Access **apple menu** ⇒ **control panels** ⇒ **extensions manager**

2. Once in **extensions manager**, you must check various extensions that may affect the serial port connection and restart computer with chosen extensions to determine if data transfer is initiated (examples: **apple share**, **apple modem**, **serial arbitrator**, and other serial devices). **NOTE**: When you restart computer **DO NOT** hold down shift key, so chosen extensions will be activated.

   NOTE: Examples of extensions that will not affect serial port connections include; **quick time**, **apple guide**, **printers**, etc.). Also, the **apple talk** program must be disabled to establish a connection. To do this, **apple menu⇒ chooser⇒ apple talk⇒** disable.

3. Once data transfer is established, save settings or note which extensions are on/off for ease in future data collection.

D. Internal Data Logging

Internal data logging is an optional feature on your Model 10-AU-005-CE. The internal data logger permits the user to record the measured sample concentration or raw data, the sample temperature, date, and time.

The sample reading is recorded as displayed on the HOME screen (3 digits). If the reading exceeds 999, the maximum for the HOME screen, then 999 will be recorded. If the reading falls below -999, the minimum for the HOME screen, then -999 will be recorded.

NOTE: If the Model 10-AU is in the auto-range mode, when it changes ranges, the readout on the HOME screen will "freeze" for 5 seconds. During this 5-second period, the "frozen" reading is the one that will be sent to the internal data logger.

If the sample temperature drops below 0°C, then "0" will be recorded (32°F). Sample temperature above 96°C (204.8°F) will be recorded as 96°C (204.8°F). Temperature will be recorded to 1 decimal: XXX.X.

The user can log up to 64,800 data points (index; date and time if applicable; sample reading; temperature if applicable):

Logging Strategy	No. Data Points
Cycle w/ temperature	18,510
Cycle w/o temperature	21,600
One Way w/temperature	43,200
One Way w/o temperature	64,800

Depending on the data logging parameters chosen, the user can log from 5.14 hours (if the interval is once a second) to 1350 days (if the interval is once every 30 minutes) before the memory is full. The factors affecting the amount of data logging time available are the strategy, the interval, and whether or not temperature is logged.

Data is retrieved by a PC through the RS-232 serial port (DB9 Female) using the fluorometer Internal Data Logger Data Output Program (IDL) provided. Using IDL, data can be converted to an ASCII format file, which can be exported to a spreadsheet program or a word processing program for manipulation.

Electronic Chart Recording is an optional feature for the internal data logger. With this feature, you can view data logged by the internal data logger on the fluorometer display without having to download it to a computer, or you can download a screen of data (240 data points) as a separate file.

If your fluorometer is equipped with internal data logging, parameters may be accessed by pressing <5> on the Main Menu. Refer to Table 7 for default values and ranges.

1. Screen 5.0: Internal Data Logger

    5.1 View data++
    5.2 Logging configuration
    5.3 Status
    Data set no. (out of 48 sets)+
    Percentage of memory left:+
    5.4 Download data to computer
    5.5 Erase data from memory

    \+ Visible only if "One Way" strategy selected
    ++ Visible only with Electronic Chart Recording

Definitions:

a. View data. If you have the optional Electronic Chart Recording capability, pressing <1> from screen 5.0, accesses screen 5.1:

```
=================================================
Strategy:
Data set no.:+
Method:                        Interval:+
Sig. unit: (PPM)               Log temp.:
Starting:+  Date    Time
Ending:     Date    Time
Press <ENT> for next data set+
Press <*> to see chart                       #5.1
=================================================
```

\+ Visible only if "One Way" logging strategy selected

This screen provides information about internal data logging parameters. If you are logging in the "ONE WAY" strategy, you can view the parameters for the different sets one by one by pressing <ENT>. The information displayed for the One Way strategy is for the set displayed under "Data set no.", not the set currently being logged.

Electronic Chart Recording (ECR). If your fluorometer is equipped with this optional feature, pressing <*> on screen 5.1 will allow you to view data logged by the internal data logger. To access the ECR, first access screen 5.3 and set data logging status to "Stop." Then, from screen 5.1, press <*>. A screen will appear, showing a data point graph:

```
==========================================================
         |<-- cursor
         |
data-->--|--^^^^_____^^____________^^^^^^^^^^^^^^^^____________
         |
         |
___________________________________________________________
<-<1> <2> <3>->   PgUp-<4>  <6>-PgDn <9>              1
11/13/91  16:30:46 = XXX.XXX 27.1 (C)
==========================================================
```

ECR Commands. The bottom line on the screen indicates the date, time, sample reading, and temperature for the data point where the cursor is presently located. There are 240 data points possible for each screen. In the Cycle strategy, if the memory is full, data will fill 78 pages (90 pages if temperature is not logged); in the One Way strategy, if the memory is full, data will fill 180 pages (270 pages if temperature is not logged). The current page number is displayed in the lower right hand corner.

Press <1> to move the cursor to the left, one data point at a time. (The cursor will wrap at the beginning of the page.)

Press <2> to move the cursor to the right, 20 data points at a time. (The cursor will wrap at the end of the page.) This function acts like the tab function in a word processing program; i.e., the movement is a fixed distance of 20 points, and it may not move 20 points if you have pressed <1> or <3>.

Press <3> to move the cursor to the right one data point at a time. (The cursor will wrap at the end of the page.)

Press <4> to go to a previous page of data.

Press <6> to advance to the next page of data. "LAST PAGE" will be displayed when the last page of data is reached.

Press <9> to send the data on the page currently displayed to an external computer. If you wish to examine a portion of the data further, you can download a selected page of the data (240 data points) to a separate file using the IDL program. (See Appendix 11F & G, below.)

Setting ECR zero and full scale points. The analog Output zero and full scale points, accessed from screen 1.4 (items 2 and 3), may be used to define the zero and full scale levels when viewing the data. For example, say the sample readouts have been ranging from 40 to 60 ppm. You could set the Output zero point to 30 on screen 1.42, and the Output full scale to 70 on screen 1.43, thus obtaining greater resolution when viewing the data.

Keep in mind that the analog outputs in this case are for viewing the data only; they have nothing to do with the internal data logger's storage of the data. They will, however, determine the zero and full scale points if you are using a regular chart recorder or other analog device; therefore, be sure to reset them before starting any analog recorder.

b. Logging configuration. From screen 5.2, you can access the following parameters:

1. Interval. The user can determine how often data will be logged into the Model 10-AU memory, from once a second to every 30 minutes.

   Total data logging time. This item appears on screens 5.2 and 5.21. It is the length of time the system will log data (with the chosen interval and options) before the memory is full. For example, if you choose a 5-second interval (in the One Way logging strategy, without logging temperature), you can log data for 90 hours before the memory is full; if you choose a 10-minute interval, you have 450 days.

   Total data logging time is actual time, not time remaining. The user-settable options that affect the logging time are: logging strategy (see subsection 3 below); data logging interval; and whether or not temperature is to be logged (see subsection 4 below).

2. Method. On screen 5.22, the user can choose either "instant" or "average". If "instant" is chosen, then data will be saved at the precise moment of the data logging interval. If "average" is selected, the data will be averaged over the past data

logging interval, and the average will be saved. For example, if 10 seconds is the data logging interval, on the "instant" setting, the reading at every 10-second mark will be saved. If "average" is selected, the reading saved at the 10-second mark will be the average of the readings in the last 10-second interval.

3. Strategy. On screen 5.23, the user can choose either "One Way" or "Cycle". One Way and Cycle are different data logging structures, so you cannot change from one to the other once data logging is started until data is erased.

   One Way. If One Way is chosen, the data will be saved in chronological order until the memory is full, then no more data will be written. A "set" of data is created each time data logging is started and stopped, with a maximum of 48 sets available. The number of sets does not change the amount of logging time available: i.e., if you have 18 hours of memory and you start logging at hour 1 and do not stop until hour 18 is over, you have used one set, but all of the memory. If you started logging at hour 1 and stopped after hour 10, started again the next day with hour 11 and stopped at the end of hour 15, you would have used 2 sets of data and 15 hours of memory. If the memory is full and you select "logging" on screen 5.3, logging status will remain at "Stop."

   Cycle. If Cycle is selected, data will be saved in chronological order until the memory is full, then the most current data will write over the old data, starting with the oldest data. Cycle uses more memory than One Way because of the need for a date and time stamp for each reading.

4. Log temperature. This item will be displayed only if the optional temperature-correction package has been purchased, and the temperature probe is in place. If so, the user can choose whether or not to save temperature data on screen 5.24. If you choose to save temperature data, it will be saved at the same interval as chosen for sample data.

c. Status. The user starts and stops data logging on screen 5.3, by selecting "stop" or "logging". If data is logging, you will not be able to change the system clock (item 4 on the Main Menu) when using the One Way logging strategy; or change the logging method (screen 5.22) or logging strategy (screen 5.23); or download to a computer (item 4, screen 5.0); or erase data (item 5, screen 5.0); or change the units of measurement (item 2, screen 1.0); or change the readout (direct vs. raw on screen 1.21). This prevents errors in data collection.

If data is logging in the Cycle strategy, the instrument will allow you to reset the system clock, although this will disrupt the continuity of date and time.

During calibration, if data is being logged, it will continue, but some data points will be nonsense. Therefore, data logging should be stopped before calibrating.

In the logging mode, "LOGGING DATA" will flash on the HOME screen under the sample reading.

If data logging is interrupted because of a power failure, data logging will resume when power is restored.

It is recommended that data logging be stopped (screen 5.3) before power to the fluorometer is shut off or disconnected. There is a very slight chance that data may be corrupted if power is interrupted while the instrument is saving data into memory.

d. <u>Download data to a computer</u>. From screen 5.4, you can download the stored data to a computer. The RS-232 cable must be connected to the computer and the computer's data collection program running, with menu item 4 or 6 selected. Then, press <8> five times to start downloading data.

Note that to avoid loss of data, you will not be able to access screen 5.4 to download data when the fluorometer is in the logging mode (access screen 5.3 to stop or start data logging).

Depending on the speed of your computer, it may take several minutes to download all data, especially if the memory is full. If downloading is interrupted before completed because of a power failure or other situation, restart the downloading procedure again. You may download data as many times as you want until it is erased.

e. <u>Erase data from memory</u>. From screen 5.5, you can erase all data from the memory. BE SURE that you have downloaded the data to a computer first (see screen 5.4). If you are sure you want to erase the data, press <9> five times. Erasure will be nearly instantaneous.

Note that to avoid loss of data, you will not be able to access screen 5.5 to erase data when the fluorometer is in the logging mode (access screen 5.3 to stop or start data logging).

Table 7. Internal Data Logging Defaults and Ranges

Data Logging Parameter	Default	Range
Data logging interval	3 (SEC)	1, 2, 3, 5, 10, 20, 30 seconds; 1, 2, 3, 5, 10, 20, 30 minutes
Total data logging time	54 (HRs)	Varies from 5 hours to 1350 days, depending on the logging options selected.
Logging Status	Stop	Stop/logging
Data set no.+	0	0 - 48
Percentage of memory left+	100%	0 - 100%
Logging method	Instant	Instant/Average
Logging strategy	One Way	One Way/Cycle
Log temperature*	No	No/Yes

\+ visible only if One Way strategy selected

* Visible only if the optional temperature-correction package has been purchased and temperature probe is plugged into the instrument

E. Using the Fluorometer Internal Data Logger Output Program (Version 1B)

The Internal Data Logger Output (IDL) is a DOS-based program written specifically to interface with the fluorometer's internal data logger. Using the program, data can be downloaded to a computer and converted to ASCII format for manipulation with standard computer programs.

Place the disk containing the IDL program in your computer.

NOTE: If the fluorometer's internal data logger memory is full, you should copy the program onto your hard disk in order to have enough memory to download and convert the data.

At the DOS prompt, load the program by typing "IDL_1B," then <ENT>. The MAIN MENU (Version 1B) will appear on your screen:

```
==========================================================
                        MAIN MENU

1.   Modify Serial Port Setup
2.   Go to DOS
3.   List directory
4.   Download Data from Instrument to File
5.   Convert Downloaded Data File to ASCII File
6.   Download and Convert Data from Instrument to File (Step
     4 + 5)
7.   Download Electronic Chart Recorder Data from Instrument
     to File
8.   View Data File Data

9.   Exit Program

==========================================================
Please enter option (1 to 9):
==========================================================
```

IDL program commands on the computer keyboard:

To select an item, press a number.

To get out of any item, press <ESC>.

To abort downloading or conversion of data, press <ESC> five times in sequence.

Press <ENT> to initiate an activity after entering a file name.

Definitions:

1. Modify Serial Port Setup. Some computers have more than one serial port. The default position is port number 1. If, however, you are already using port number 1 for a mouse or other serial device, then change to port number 2 by pressing <1> on your computer, then pressing <2> to select port number 2.

   If the wrong port is selected, during downloading "COMMUNICATION ERROR" will appear on the fluorometer screen. Abort the downloading procedure, select the correct port, and initiate the downloading sequence again.

2. Go to DOS. By pressing <2> on your computer keyboard, you can go to DOS and enter DOS commands. This is a convenient feature if, for example, you want to check the directory or erase files to free additional memory.

3. List directory. If you press <3> on your computer keyboard, you will be prompted to enter the name of a directory for viewing the files in that directory. For example, if you enter C:\dirname, a list of the files in the directory called "dirname" on the C drive will appear. Press <ENT> to see a list of the files in the current directory. You can also use a wildcard to list only the "PRN" or "PLT" files: i.e., *.PRN will list only the files with a PRN extension. After a directory has been listed, press <ESC> to return to the IDL program.

4. Download Data from Instrument to File. Pressing <4> on the keyboard accesses the downloading sequence. In this step, data is transferred from the fluorometer to the computer. It is saved in a binary (BIN) compressed data file, which is not in a readable or printable form until it is converted into ASCII format (menu item 5). The reason for the BIN file is to save disk space and downloading time.

   During step 4, after the data is downloaded from the fluorometer, IDL will prompt you for a file name: a maximum of eight-characters and a period, followed by the extension "BIN." You do not have to type the period or BIN; it will be entered automatically, unless you enter a different extension. It is strongly recommended that you use the "BIN" extension to distinguish these files from readable ASCII files. If you like, you can take the default name "IDATALOG.BIN" by pressing <ENT>.

   The data will automatically be saved on the drive where the program is located and opened, unless you enter a different drive path. For example, if you opened IDL from a floppy in drive A, and you wish to save data to the hard drive C, then when prompted to name the file, type "C:\name of directory\xxxxxxxx (the file name)" and press <ENT>. The file will then be saved on drive C in the directory named.

If the fluorometer memory is full, the minimum disk space required for downloading alone (separate from the program itself) is 130 KBytes, independent of data logging parameters chosen.

When downloading is in process, "Serial Communication Error" will appear on the computer screen after several seconds if:

- The power to the fluorometer fails;
- The RS-232 cable is disconnected;
- <ESC> is pressed on the fluorometer keypad (aborts the downloading sequence).

When downloading is in process, "COMMUNICATION ERROR" will appear on the fluorometer display if:

- The wrong serial port is selected (item 1);
- The RS-232 cable is disconnected;
- <ESC> is pressed 5 times in sequence on the computer (aborts the downloading sequence).

If downloading is interrupted for any reason, simply return to the Main Menu on the computer and screen 5.4 on the fluorometer and rerun the downloading sequence.

If speed is important to you, you are advised to use at least a 286 or higher model computer. For example, if the fluorometer's internal data logger memory is full, it may take as long as 9 minutes for data to be downloaded using an XT-type computer.

The fluorometer will display the number of data blocks downloaded. Each data block is 1 KByte, with a maximum of 127 blocks possible.

5. <u>Convert Downloaded Data File to Ascii File</u>. This step takes the BIN file downloaded from the fluorometer and converts it to an ASCII file, which can be retrieved, read, and manipulated using a spreadsheet program or a word processing program.

   Pressing <5> accesses the ASCII conversion sequence. You will be prompted to enter the downloaded (BIN) data file to be converted. Then you will be asked to give a name to the ASCII file: an eight-character name and a period, followed by the suffix (extension) "PRN." You do not have to type the period or PRN; it will be entered automatically, unless you enter a different extension. It is strongly recommended that you use the "PRN" extension, as it is recognized by DOS as a printable file. If you like, you can take the default name by pressing <ENT>. Note that if you have just downloaded a file, the default name will be the same as the name for the BIN file including the drive path, except it will have a "PRN" extension.

If you wish to save the converted file on a different drive, then enter a different drive path. For example, if you are using a floppy in drive A, and you wish to save data to the hard drive C, then when prompted to name the file, type "C:\name of directory\xxxxxxxx (the file name)" and press <ENT>. The file will then be saved with a PRN extension on drive C in the directory named.

When naming is complete, IDL will automatically begin converting the BIN file to an ASCII file. The percentage of data converted will appear in the lower right-hand corner of the computer screen. If conversion is interrupted, simply begin the conversion sequence again.

If speed is important to you, you are advised to use at least a 286 or higher model computer. For example, if the fluorometer internal data logger memory was full, it may take as long as 12 minutes for conversion to an ASCII file with an XT-type computer.

A great deal of disk memory will be required to save a full memory: for 18,000 points, over 900 KBytes may be required.

6. Download and Convert Data from Instrument to File. This step allows downloading and conversion to take place as a single step. The memory considerations and time factors for steps 4 and 5 above apply to step 6.

   To download and convert as one step, press <6> on the computer. Then access screen 5.4 on the fluorometer and press <8> five times to start downloading. When it is completed, the IDL will prompt you to name the "BIN" file. "Data Retrieval Completed" will appear, and IDL will ask you to give a name to the new ASCII file. Type the name and press <ENT>, or press <ENT> to take the default, and IDL will convert the data to an ASCII file.

7. Download Electronic Chart Recorder Data from Instrument to File. If your fluorometer is equipped with the optional Electronic Chart Recording (ECR), menu item <7> allows you to download a page of the data (240 data points) from the internal data logger.

To download a page of data, access the ECR screen by pressing <*> from screen 5.1. Page through the data until the page of data you wish to download is displayed. Then press <7> on your computer and <9> on the fluorometer. When downloading is completed, the IDL will prompt you to name the file. Type the name and press <ENT>, or press <ENT> to take the default ("ECR.PLT"), and IDL will save the data (with a "PLT" extension) in ASCII format. The "PLT" extension will help distinguish files downloaded from the ECR from other PRN files. When saving is complete, IDL will display "Data Retrieval Completed."

The information sent to the computer includes: data about internal data logging parameters (from screen 5.1), date, time, sample readout, and temperature. The data points will also be graphed with a line of asterisks (one for each data point) in a rectangular grid.

Once <9> is pressed, for the first 20 seconds none of the functions on the ECR screen will operate until the data is downloaded from the fluorometer. Then, depending upon the speed of your computer, it may take as long as a minute for the computer to process the data. To abort the downloading procedure, press <ESC> on the fluorometer or computer.

Once a file is downloaded from the ECR, it can be printed, viewed, or imported into a word processing or spreadsheet program for manipulation. See Appendix 11G.

8. <u>View Data File Data</u>. This item allows a "PRN" or "PLT" file, or any text file (ASCII file with a maximum of 80 characters per line), to be accessed and viewed a page at a time. Press <8> and IDL will prompt you for the name of the file to be viewed. Enter the name, including any drive path specification, a period and the extension "PRN" or "PLT" (or other extension if applicable). The file will appear on the screen. To move through the data a line at a time, press <L>. Press any key to page down through the data. When the end of the file is reached, IDL will display "Press <ESC> to return to IDL program." To return to IDL before completely viewing a file, press <Q>.

   If you can't remember the name of a file you want to view, check by accessing the "List Directory" function, item 3 on the Main Menu, before entering the view data file function.

Running IDL:

This procedure is for downloading and converting in two steps (steps 4 and 5 of IDL). If you wish to use step 6, the combined method, the procedure is basically the same except in step 6 below, enter <6>; and you will do steps 8 - 10 below as a single step. If you wish to download a page of data points from the ECR to a separate file, refer to the section "Running IDL with ECR," immediately following these instructions.

1. Connect your computer to the fluorometer's RS-232 serial port (a DB9 female connector on the power/telemetry connector).

2. Load the IDL program by typing "IDL_1B" (for Version 1B) at the DOS prompt (C:\ or A:\, etc.). The Main Menu will appear.

3. Access screen 5.0 on the fluorometer. From the Main Menu, press <5>.

4. If data is currently being logged, press <3> to access screen 5.3 and set the logging status to "Stop."

5. From screen 5.0, press <4> to bring up screen 5.4.

6. On the computer keyboard, press <4> to access the downloading procedure.

7. On the fluorometer keypad, press <8> five times and data will be downloaded. The fluorometer will display the number of blocks of data downloaded. Depending on the speed of your computer, it will require from 2 to 9 minutes for downloading if the fluorometer memory is full.

8. When downloading is finished, IDL will ask you to name the downloaded file. Type in a maximum of eight characters for the name. (If you wish to change the drive path, enter the drive, followed by the name of the file, i.e.: "C:\name of directory on C drive\name of file".) The name will appear with the "BIN" extension, <u>unless</u> you enter a different one. It is strongly recommended that you use the "BIN" suffix for all downloaded files to distinguish them from the readable ASCII files (step 5 on the IDL Main Menu). Press <ENT> on the computer keyboard to take the name chosen or the default name "IDATALOG.BIN." The computer will display "Data Retrieval Completed".

   If you would like to wait until later to convert the data to ASCII format in order to save disk space, then skip to step 11.

   To convert the data to ASCII, proceed with step 9.

9. To convert a BIN file to an ASCII file, press <5> on the computer keyboard. IDL will then ask you to name the file to be converted to an ASCII file (the name of the BIN file you last downloaded or the default name will appear). Press <ENT> to take the default, or type in the name of the BIN file you wish to convert (including any drive path specified) and press <ENT>.

10. IDL will then ask you to name the new ASCII file. Type in a maximum of eight letters. (If you wish to change the drive path, enter the drive, followed by the name of the file, i.e.: "C:\name of directory on C drive\name of file".) The name will appear with the "PRN" extension, <u>unless</u> you enter a different one. It is very important to use the "PRN" suffix because it is commonly used in DOS-based programs to identify a printable file. This extension will also help distinguish between the "BIN" files and the converted files. Press <ENT> on the computer keyboard to take the name chosen or the default.

    If the BIN file is found and the computer is able to open the PRN file, the compressed data from the BIN file will be converted to an ASCII file. The percentage of data converted will appear in the lower right-hand corner of the computer screen.

11. When finished, press <9> to exit IDL.

12. Disconnect the computer from the fluorometer. Erase the data currently in the fluorometer and enter new internal data logging parameters if desired, or resume logging with the previous parameters (see Appendix 11E). Return the fluorometer to normal operation.

Running IDL with ECR:

1. Follow steps 1 - 4 in the previous section, "Running IDL."

2. From screen 5.0, press <1> to bring up screen 5.1, then press <*> to call up the electronic data chart.

3. Page through the data until the page of data you want to download is displayed.

4. On the computer keyboard, press <7> to access the downloading procedure.

5. On the fluorometer keypad press <9>. It may require a minute or so to process the data, depending upon the speed of your computer. You will not

be able to access ECR functions on the keypad during the first 20 seconds. Press <ESC> on the fluorometer or computer to abort the downloading.

6. When downloading is finished, IDL will ask you to name the downloaded file. Type in a maximum of eight characters for the name. (If you wish to change the drive path, enter the drive, followed by the name of the file, i.e.: "C:\name of directory on C drive\name of file".) The name will appear with the "PLT" extension, unless you enter a different one. It is strongly recommended that you use the "PLT" extension, as this will distinguish ECR files from other IDL "PRN" files. Press <ENT> on the computer keyboard to take the name chosen or the default name (ECR.PLT). The computer will display "Data Retrieval Completed".

7. Download another page of data, if desired; or, press <9> to exit IDL.

8. Disconnect the computer from the fluorometer. Erase the data currently in the fluorometer and enter new internal data logging parameters if desired, or resume logging with the previous parameters (see Appendix 11E). Return the fluorometer to normal operation.

F. Examining the Downloaded Data (from either external or internal serial data logging)

The ASCII-format "PRN" or "PLT" files can be opened, viewed, or printed using typical DOS commands.

For your convenience, we have provided you with a "browse" program, which will allow you to examine any ASCII file using various cursor keys. (See "Viewing a file with the Browse Command," below.)

If you have been logging data for some time, you will have a lot of data to examine and it is suggested that you consider editing your file (see Editing a File, below) before you do anything else.

NOTE: If speed is important to you, it is recommended that you use a 286 or higher model computer. An XT-type will work, but it will be very slow.

A typical line of data from the internal data logger will look like this:

```
00001: 10/24/91  14:10:28 = 11.300  98.6 (F)
```

The first column is an index number; the second is the date; the third, the time; the fourth, the sample readout (as reflected on the HOME screen); and the final column indicates the sample temperature, available only if your fluorometer is equipped with temperature compensation and you choose to log temperature.

Opening a File. To open the file, at the DOS prompt enter TYPE and the file name, i.e.: "TYPE xxxxxxxx.PRN. This command, however, will open the file and the computer will rapidly scroll through the data.

Viewing a File with the Browse Command. The "browse" command allows you to view any ASCII file at your own pace using the cursor arrows, Page Up, Page Down, Home, and End commands. To view a file in this way, type BROWSE and the file name, i.e., "BROWSE xxxxxxxx.PRN".

Printing a File. To print a file, at the DOS prompt, type PRINT and the file name, i.e.: "PRINT xxxxxxxx.PLT". This will print the entire file; keep in mind that if you have been logging data for some time, this might be several hundred pages.

Viewing a File. To view a file a page at a time, at the DOS prompt type MORE < and the file name, i.e.: "MORE < xxxxxxxx.PRN". You can then use any key to page down through the data. Again, if you have been logging data for some time, you may have several hundred pages of data to scroll through. To escape from viewing, press <Ctrl C> or <Ctrl Break>.

Note that you can also view a file using the IDL program (menu item 8).

Editing a File. To edit a file, the data can be imported into a word processing program or a spreadsheet program.

Note that if you have Internal Data Logging and the optional Electronic Chart Recording, you can download a page of data (240 data points) to a separate file. This is a very manageable file size for manipulation of data.

Using Word Perfect©. If the fluorometer's internal data logger memory was full, you probably have a PRN file exceeding 900 KBytes. This file can be opened as a word processing file.

For example, using Word Perfect, the entire file can be opened, examined, and edited to a more manageable size. With a full memory, a file will cover some 18,500 - 64,800 lines and several hundred pages.

Open the file as a DOS text file (without conversion); this will allow for editing and will be much faster than converting a text file to Word Perfect. For example, with an XT-type computer, it may take 5 minutes to load the whole file as a text file; to convert the file to a Word Perfect file, it might take more than 30 minutes.

To open it as a DOS text file, open a new Word Perfect file, press <Ctrl F5> to access the "Text In/Out" function, then press item <1> DOS; then <3>

Retrieve (CR/LF to [SRt] in HZone" to retrieve the document in DOS text. You can then edit the file to contain only the data of interest to you: select various pages; eliminate repetitive data, etc. Or, you could print the entire file or selected pages.

If you intend to import the data into a spreadsheet program, it is recommended that you edit the file to 8000 lines or less. Lotus 123©, for example, will accept only the first 8,192 lines of a PRN file.

To import to a spreadsheet program, you must save the word processing file as a DOS text file.

Using Lotus 123©. If the fluorometer's internal data logger memory was full, only the first 8,192 lines of the PRN file can be imported into Lotus. A slow computer (4.77 MHz XT without a math processor) can take more than an hour to convert the first 8,192 lines of data, and about 853 KBytes of memory will be required.

Thus, when dealing with a large file, it is recommended that you edit the file first using a word processing program. Open/save the edited word processing file as a DOS text file and then import it into Lotus. (Lotus commands: Worksheet; File; Import; Numbers; name of file to be imported (including the "PLT" extension, if applicable.)

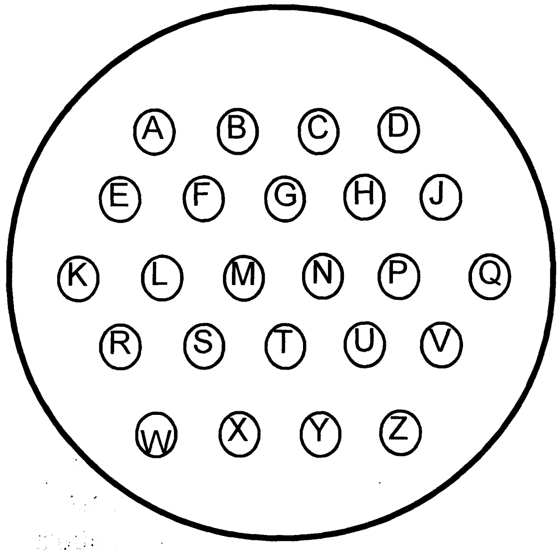
Using Microsoft Excel®. Data "PRN" files can be imported into Excel® (version 4.0 or greater) running on Windows©. You can use the "column delimiter" function to organize the data in the proper columns. To use the column delimiter function:

1. Start Windows running.
2. Open Excel program.
3. Under "File", select "Open".
4. Dialog box appears. Select the drive your "PRN" file is on. Under "List files of type", select *.*, i.e., all types. Your PRN file should appear.
5. While still in the dialog box, open the "Text..." dialog box. Under "column delimiter", select "Space".
6. Select and open your PRN file and it will feed into the proper columns.

G. Power\Telemetry Connector Pin Inputs and Outputs

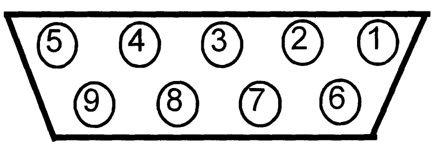
Power/Telemetry Connector

(Male pins, receptacle mounted on instrument, front view)



RS-232 Serial Connector

(DB9 female, front view)



RS-232 Serial Output

PIN	CONNECTION
W	6+8 (data set ready)
X	2 (transmit)
Y	3 (receive)
Z	5 (signal ground)

Power Input

PIN	CONNECTION
K	Negative (12 VDC)
L	Positive (12 VDC)
N	Green (chassis ground)

Analog Output (DC volts)

PIN	CONNECTION
U	Black (negative)
V	Red (positive)

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GLOSSARY

Term	Definition
Auto-ranging	The Model 10-AU can be set to choose the optimum range for the sample being read. This "auto-ranging" function allows both low and high concentrations to be read with the optimum resolution. See Section 2G, subsection 2 and screen 2.43.
Chopper Motor Speed	In the diagnostic mode, chopper motor speed refers to the speed of the chopper motor which is used in the light path to stabilize the fluorometer. A motor speed problem can typically be repaired only by returning the instrument to the factory. See Section 4 and Appendix 5B.
Circuit Failure	In the diagnostic mode, a circuit failure alarm refers to a breakdown on one of the printed circuit boards located in the fluorometer. This failure can typically only be cured by returning the instrument to the manufacturer. See Section 4.
Continuous-flow Cuvette/Cell	A glass or quartz tube, housed in a Delrin body, through which sample flows for reading on a continuous basis. (Appendix 7.)
Cuvette	The glass or quartz test tube which holds the sample through which the light from the lamp shines. The cuvette is held by the proper size cuvette holder. See Appendix 7.
Emission Filter	A filter located between the cuvette and the photomultiplier window. It may be encased in the emission filter holder. Its function is to pass only wavelengths of light particular to your measurements to the light detector. See Appendices 2 and 8.

Term	Definition
Excitation Filter	A filter(s) particular to your application located between the lamp and the flow cell or cuvette. Its function is to transmit wavelengths of light particular to your application. See Appendices 2 and 8.
Fluorometer Malfunction Alarm	An alarm activated when the system senses a malfunction within the fluorometer. See Section 4.
Span	A function used to fine tune the sensitivity during calibration. Using the keypad, the operator increases or decreases Span on screens 2.11 and 2.3 when calibrating the instrument. See Section 2G.
High Voltage	In the diagnostic mode, this refers to the voltage to the photomultiplier tube. The high voltage controls the sensitivity of the photomultiplier tube. A high voltage failure can typically only be repaired by returning the instrument to the manufacturer. See Section 4 and Appendix 5B.
High Level Alarm	An alarm activated when the sample reads above the user-set level for triggering this alarm. See Section 4 and Appendix 5A.
Lamp	A six-inch fluorescent tube-type lamp, which is housed in the front of the fluorometer and excites the sample with light passed through the excitation filter. This lamp should not be viewed directly as it will cause a sunburn condition to your eyes. A lamp failure can usually be cured by replacing the lamp. See Section 4 and Appendix 8.
LCD	This is the liquid crystal display, the digital display mode used on the fluorometer. See Section 2B.

Term	Definition
Range (Concentration Range)	The operator sets the concentration range in increments of 10 (Low, Med, High) during calibration on screen 2.42. See Section 2G. (See also Auto-ranging, above.)
Sensitivity Adjustment Knob	A control located at the lower right side of the keypad that sets the overall operating level (sensitivity) of the fluorometer. It is locked with a hex nut located to the left of the keypad. Before calibrating your instrument for the first time, you must set the operating level for your application using this knob (see Appendix 6B). Thereafter, DO NOT ADJUST the Sensitivity Adjustment Knob, unless you change to a different kind of lamp or filters, or a new cuvette size.
Temperature Probe	If you have purchased the optional temperature-compensation package, your Model 10-AU will have a temperature probe. This is a sensor that detects the temperature of the sample in the flow cell (it will not operate with the cuvette holder) for use in temperature correction. For more information on temperature parameters, see Appendix 5A, screen 1.7.

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APPENDIX G

YSI Model 63 Handheld pH, Conductivity, Salinity, and Temperature Operator's Manual

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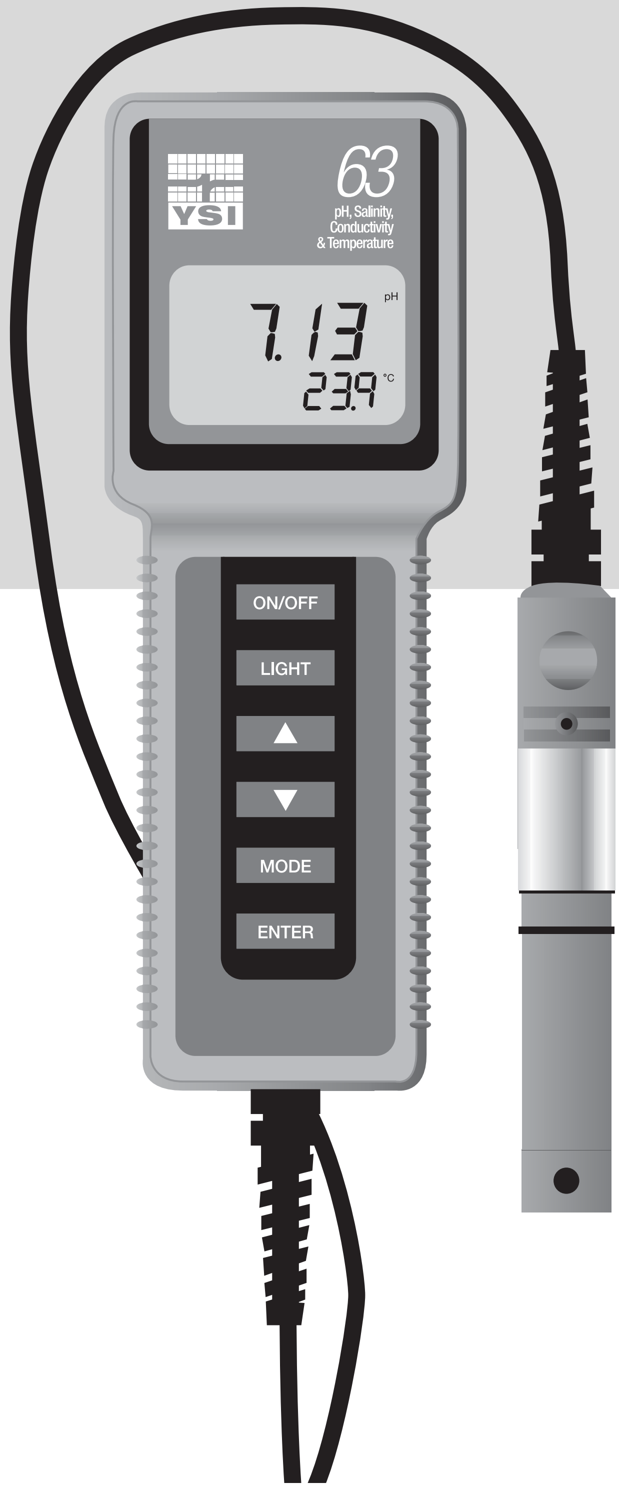
Y S I *incorporated*



YSI Model 63

Handheld pH, Conductivity, Salinity and Temperature System

Operations Manual

Table of Contents

1. Introduction

The YSI Model 63 Handheld pH, Conductivity, Salinity and Temperature System is a rugged, micro-processor based, digital meter with an attached pH, conductivity and temperature probe. The pH sensor can be easily replaced in the field.

The Model 63 has a non-detachable cable available in lengths of 10, 25, 50 or 100 feet (3, 7.6, 15.2 or 30.5 meters). The probe body has been manufactured with stainless steel to add rugged durability and sinking weight.

The YSI Model 63 has the following features:

- Capability to measure at depths of up to 100 feet (30.5 meters)
- Microprocessor control
- Field replaceable low maintenance pH sensor
- Push-button calibration
- Simultaneous display of pH, conductivity or salinity and temperature
- Automatic temperature compensation for conductivity readings
- Autoranging
- Data storage for 50 sets of readings with on screen recall
- Waterproof case (IP65)

The Model 63's micro-processor allows the system to be easily calibrated with the press of a few keys. Additionally, the micro-processor performs a self-diagnostic routine each time the instrument is turned on. The self-diagnostic routine provides useful information about the function of the instrument and probe.

A transport chamber, built into the instrument case, provides a convenient place to store the probe when transporting the system. The Model 63 case is waterproof (rated to IP65) allowing operation in the rain without damage to the instrument.

The Model 63 is powered by six AA-size alkaline batteries. A new set of alkaline batteries will provide approximately 100 hours of continuous operation. When batteries need to be replaced, the LCD will display a **"LO BAT"** message.

The YSI Model 63 is designed for use in environmental, aquaculture, and industrial applications where accurate pH, conductivity, salinity and temperature measurements are desired.

2. Preparing the Meter

2.1 Unpacking

When you unpack your new YSI Model 63 Handheld pH, conductivity, salinity and Temperature System for the first time, check the packing list to make sure you have received everything you should have. If there is anything missing or damaged, call the dealer from whom you purchased the system. If you do not know which of our authorized dealers sold the system to you, call YSI Customer Service at 800-765-4974 or 937-767-7241, and we'll be happy to help you.

2.2 Warranty Card

Please complete the Warranty Card and return it to YSI. This will record your purchase of this instrument in our computer system. Once your purchase is recorded, you will receive prompt, efficient service in the event any part of your YSI Model 63 should ever need repair.

2.3 Batteries

There are a few things you must do to prepare your YSI Model 63 for use. First, locate the six AA-size alkaline batteries and the battery cover kit which were included. Then locate the markings inside each of the two battery-chamber sleeves that illustrate the correct way to install the batteries. Install the batteries are shown.

NOTE: It is very important that the batteries be installed ONLY as illustrated. The instrument will not function and may be damaged if the batteries are installed incorrectly.

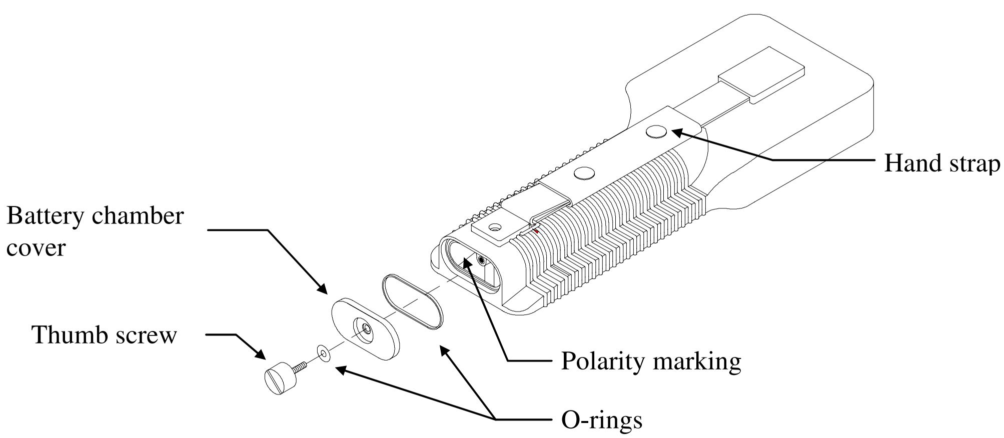


Figure 1

Attach the battery chamber cover to bottom of the instrument using the thumb screw as shown in *Figure 1*. Make sure that the o-rings are in place. The battery-chamber cover is marked with the words "OPEN" and "CLOSE."

Turn the instrument on by pressing and releasing the **ON/OFF** key on the front of the instrument. The liquid crystal display (LCD) should come on. Allow a few seconds for the instrument to complete its diagnostic routine. If the instrument does not operate, consult the chapter entitled *Troubleshooting*.

You may also want to take the instrument into a dark location and, with the instrument ON, hold down the **LIGHT** key. The instrument back-light should illuminate the LCD so that the display can be easily read.

2.4 Transport Chamber

The Model 63 has a convenient transport chamber built into the instrument's side. This chamber provides a storage area and protection for the probe while transporting the system in the field. Insert the round sponge (provided with the Model 63) into the bottom of the chamber. Put 6-8 drops of tap water into the sponge. The wet sponge creates a humid environment for the pH sensor to prevent it from drying out during transport in the field (up to one week). The transport chamber is NOT intended for long term storage of the pH sensor. See *6.3 pH Sensor Storage*.

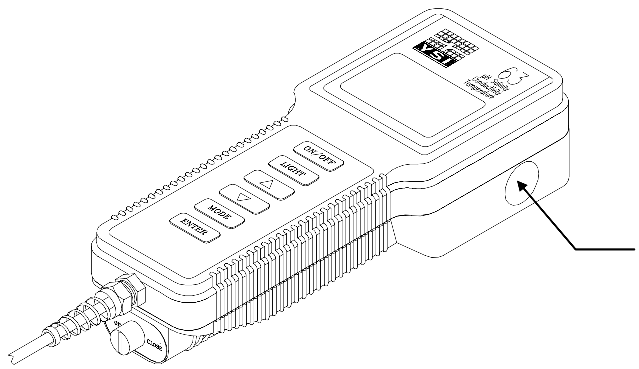


Figure 2

2.5 Hand Strap

The hand strap (see Figure 1 on previous page) is designed to allow comfortable operation of the Model 63 with minimum effort. If the hand strap is adjusted correctly, it is unlikely that the instrument will be easily dropped or bumped from your hand.

To adjust the hand strap on the back of the meter, unsnap the vinyl cover and pull the two Velcro strips apart. Place your hand between the meter and the strap and adjust the strap length so that your hand is snugly held in place. Press the two Velcro strips back together and snap the vinyl cover back into place.

2.6 The Meter Case

The meter case is sealed at the factory and is not intended to be opened, except by authorized service technicians. **Do not attempt to separate the two halves of the meter case as this may damage the instrument, break the water-resistant seal and may void the manufacturer's warranty.**

2.7 Calibration Vessels

To do a calibration you will need a plastic 100 mL graduated cylinder. A plastic container is provide with the Model 63. The graduated cylinder provides a convenient place to calibrate the pH sensor minimizing the amount of solution needed. The plastic container can be used as a conductivity calibration vessel or filled with distilled water and used as a rinse vessel while in the field. See section *4.2 pH Calibration* and section *4.3 Conductivity Calibration* for details.

3. Preparing the Probe

The YSI Model 63 is shipped without the pH sensor installed. The pH sensor must be installed before using the system (see section *3.1 Installing the pH Sensor*, below). The sensor is shipped with a protective bottle filled with a mixture of pH 4 buffer and KCl solution. Do not remove the bottle until you are ready to use the instrument. Save the bottle for long term storage of the probe.

3.1 Installing the pH Sensor

A pH sensor is included with the Model 63. Install the pH sensor as follows:

1. Remove the sensor from its protective packing.
2. Insert the pH sensor into the probe body (be sure to align the tabs on the sensor with the slots in probe body) and twist 1/4 turn to lock in place. See *Figure 3*.

NOTE: Once installed, leave the pH sensor attached to the probe until replacement is needed.

3. Carefully remove the protective bottle (containing pH 4 buffer/KCl solution) from the sensor. Save the bottle and solution for long term (more than 1 week) storage of the sensor. Seal the storage bottle with the cap provided.
4. Rinse the sensor tip with distilled or deionized water.
5. **Calibrate the system before use.** See section *4.2 pH Calibration*.

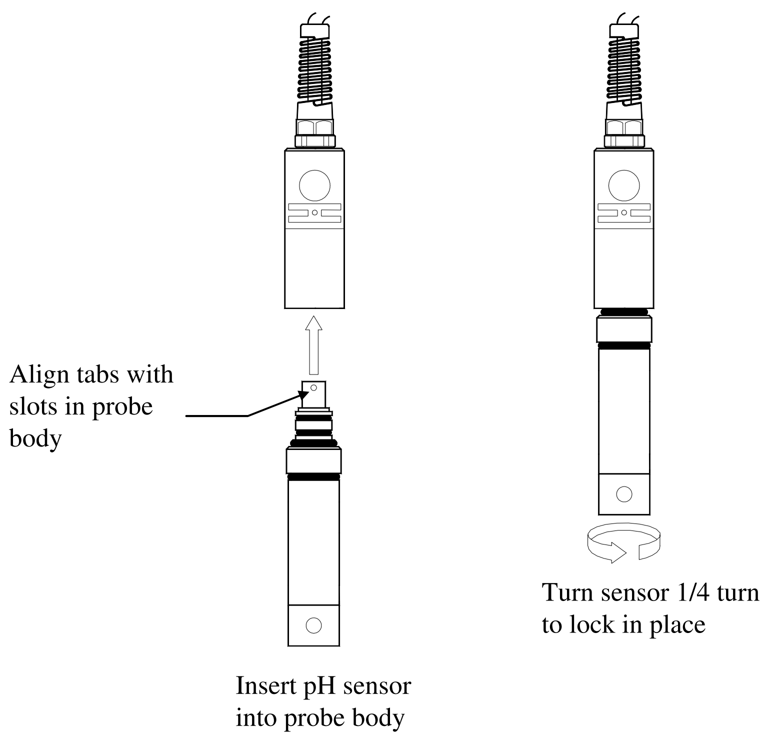


Figure 3

4. Operation

The following diagram is an overview of the operation of the Model 63. See the following sections for details of operation.

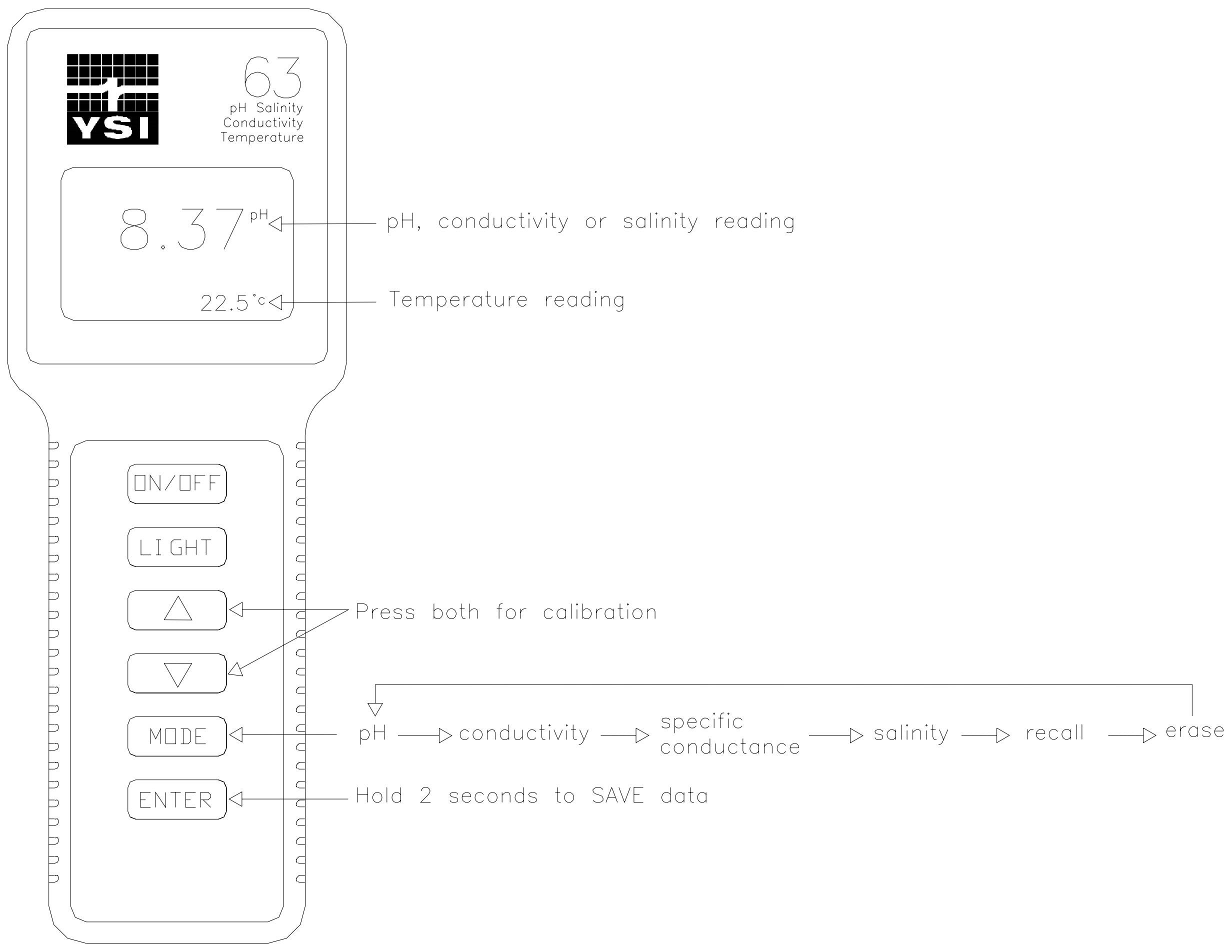
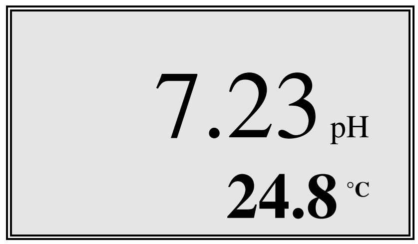


Figure 4

4.1 Turning The Instrument On

With the batteries installed correctly, press the **ON/OFF** key. The instrument will activate all segments of the display for a few seconds, which will be followed by a self test procedure which will last for several more seconds. The Model 63 will briefly display the cell constant of the conductivity probe when the self test is complete. During this power on self test sequence, the instrument's microprocessor is verifying that the system is working properly. If the instrument were to detect a problem, a **continuous** error message would be displayed. See the chapter entitled *Troubleshooting* for a list of error messages.



4.2 pH Calibration

The YSI Model 63 ***MUST*** be calibrated before making pH measurements. Calibration may be performed at 1, 2 or 3-points (at pH 7, 4 and 10, or at pH 6.86, 4.01 and 9.18). Perform a 1-point calibration (at pH 7 or at pH 6.86) ***ONLY*** if a previous 2 or 3-point calibration has been performed recently. In most cases, a 2-point pH calibration will be sufficient for accurate pH measurements, but if the general range of pH in the sample is not known, a 3-point calibration may be necessary. 3-point calibration assures accurate pH readings regardless of the pH value of the sample. See *9.1 pH* for more details.

WARNING: Calibration reagents may be hazardous to your health. Refer to *Appendix B - Health and Safety* for more information.

Before calibrating the YSI Model 63, complete the procedures discussed in the *Preparing the Meter* and *Preparing the Probe* chapters of this manual.

The user can choose from two sets of pH buffer values for 3-point calibration. The first set consists of the standard YSI pH buffer values of pH 7 (YSI 3822), pH 4 (YSI 3821) and pH 10 (YSI 3823). The second set available is the NIST pH 6.86, 4.01 and 9.18. **Note that the first calibration point must be either pH 7 or pH 6.86**. Calibration is performed as follows:

1. Turn the instrument on by pressing the **ON/OFF** key. Press the **MODE** key until pH is displayed.

2. Rinse the probe with deionized or distilled water, then carefully dry the probe (or rinse it with some of the pH buffer solution to be used for calibration).

3. Place 30 to 35 mL of the pH buffer you have chosen to calibrate the system with (pH 7 or 6.86) in the 100 mL graduated cylinder. The graduated cylinder minimizes the amount of solution needed. Immerse the probe making sure that both the pH and temperature sensors are covered by the solution (see *Figure 5* on the following page).

For best results:

- Calibrate as close as possible to the sample temperature.
- After storage in pH 4 buffer/KCl solution, place the pH sensor in pH 7 (6.86) buffer and allow to acclimate before calibrating (5 to 10 minutes).
- Always give the pH and temperature sensors enough time to equilibrate with the temperature of the buffer.

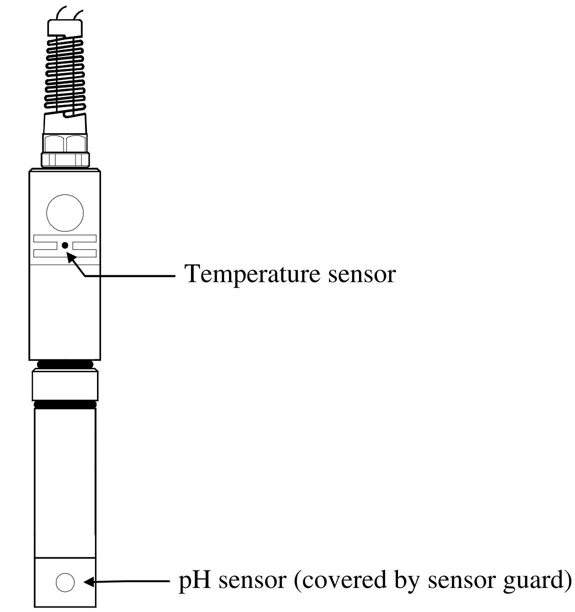
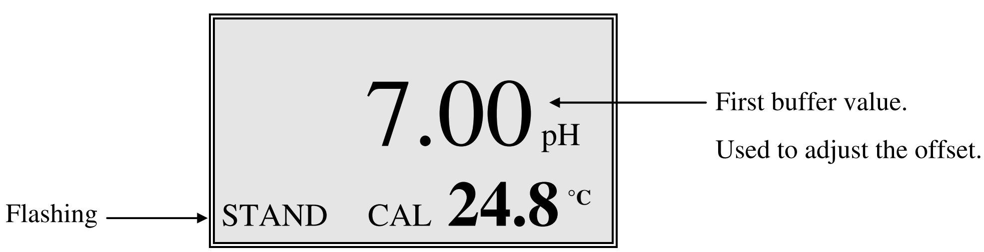


Figure 5

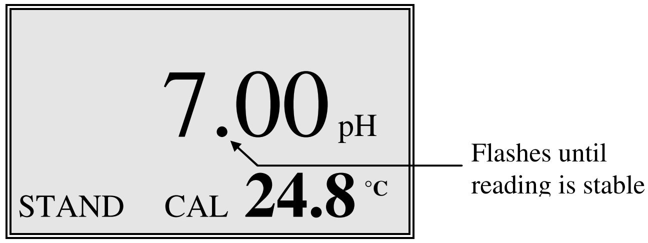
4. To enter the calibration menu, use two fingers to press and release both the **UP ARROW** and **DOWN ARROW** keys at the same time. The Model 63 display will show **CAL** at the bottom, **STAND** will be flashing and the pH reading will show **7.00** (the buffer to be used to adjust the offset).



NOTE: If you will be calibrating with pH buffers of 6.86, 4.01 and 9.18 (instead of 7, 4 and 10), press both the **UP ARROW** and **DOWN ARROW** keys again. The display will change to **6.86**.

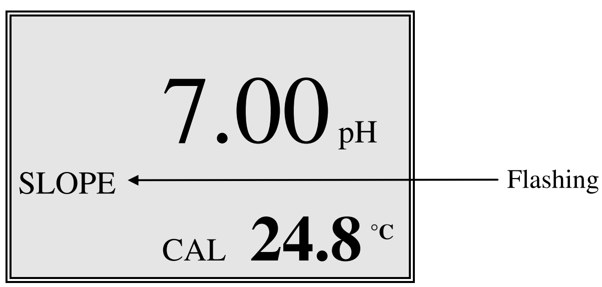
NOTE: The Model 63 automatically accounts for the fact that the true pH of the buffers changes with temperature, therefore, the pH values displayed during calibration will vary with temperature. For example, pH 7 buffer at 20°C (rather than 25°C) has an actual pH of 7.02 and this number (rather than 7.00) will appear on the display when the probe is placed in the solution. See *Appendix C - pH Buffer Values*.

5. Press the **ENTER** key. The Model 63 display will show **CAL** at the bottom, **STAND** will stop flashing and the pH calibration value is shown with the middle decimal point flashing.



6. When the reading is stable (does not change by 0.01 pH in 10 seconds), the decimal point will stop flashing. Press and hold the **ENTER** key to save the calibration point. The Model 63 will flash **SAVE** on the display along with **OFS** to indicate that the offset value has been saved.

7. **SLOPE** will now appear on the display and be flashing. This indicates that the slope is ready to be set using a second pH buffer. The system is now calibrated at a single point. If you are only performing a single point calibration, press the **MODE** key to return to normal operation.

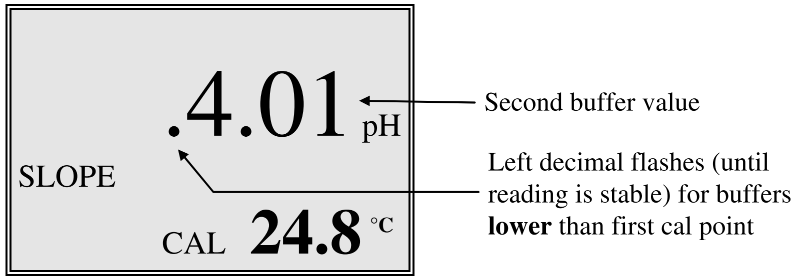


8. Rinse the probe with deionized or distilled water, then carefully dry the probe.

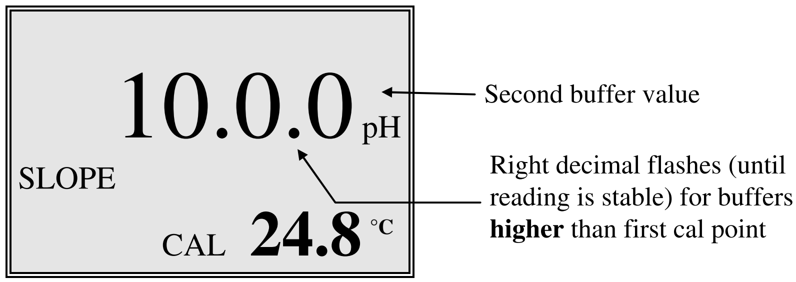
STOP HERE IF PERFORMING A 1-POINT CALIBRATION.

9. If you are performing a 2-point or 3-point calibration, fill a clean container with the second value pH buffer (pH 4 or 10, or pH 4.01 or 9.18) and immerse the probe into the solution. Make sure that the temperature sensor is immersed.

10. Press the **ENTER** key. The Model 63 should now show **CAL** at the bottom, **SLOPE** will stop flashing and the pH calibration value (automatically sensed by the instrument) is shown with one of the decimal points flashing.

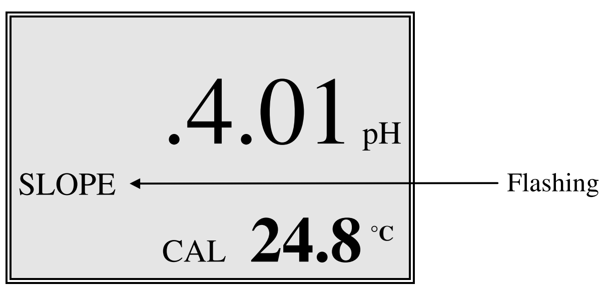


If the second pH buffer is less than the first buffer (which was used to adjust the offset; pH 7 or pH 6.86), the left decimal point will flash as shown above. If the second pH buffer is greater than the first, the right decimal point will flash as shown below.



11. When the reading is stable (does not change by 0.01 pH in 10 seconds), the decimal point will stop flashing. Press and hold the **ENTER** key to save the first SLOPE. The Model 63 will flash **SAVE** on the display along with **SLP** to indicate that the first slope value has been saved.

12. **SLOPE** will start flashing again indicating that the slope is ready to be set using a third pH buffer.



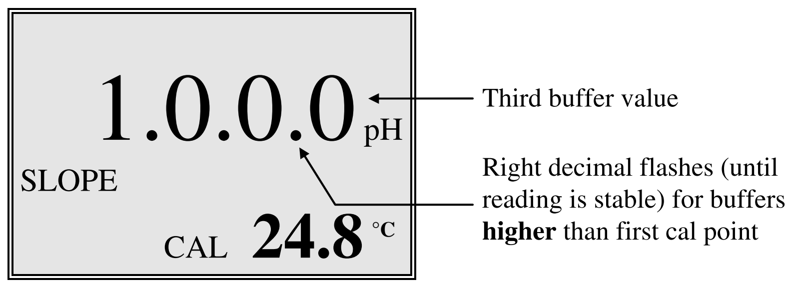
13. The system is now calibrated at two points. If you are only performing a two point calibration, press the **MODE** key to return to normal operation.
14. Rinse the probe with deionized or distilled water, then carefully dry the probe.

STOP HERE IF PERFORMING A 2-POINT CALIBRATION.

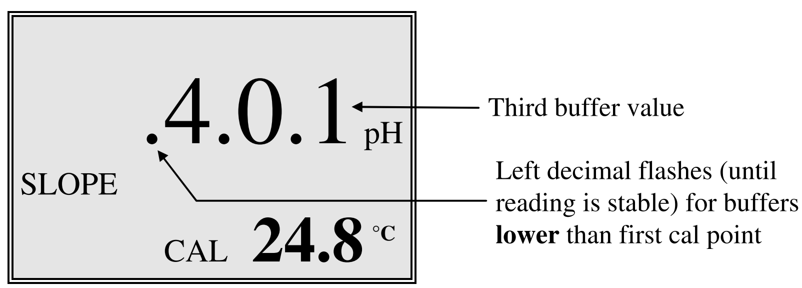
15. If you are performing a 3-point calibration, fill a clean container with the third value pH buffer (pH 4 or 10, or pH 4.01 or 9.18) and immerse the probe into the solution. Make sure that the temperature sensor is immersed.

NOTE: The third buffer must not be the same as the second buffer. For example; if the second buffer was less than pH 7, the third buffer must be greater than pH 7.

16. Press the **ENTER** key. The Model 63 display will now show **CAL** at the bottom, **SLOPE** will stop flashing and the pH calibration value (automatically sensed by the instrument) is shown with one of the decimal points flashing. If the third pH buffer is less than the first buffer (which was used to adjust the offset; usually pH 7), the left decimal point will flash. If the third pH buffer is greater than the first, the right decimal point will flash.



or



17. When the reading is stable (does not change by 0.01 pH in 10 seconds), the decimal point will stop flashing. Press and hold the **ENTER** key to save the second SLOPE. The Model 63 will flash **SAVE** on the display along with **SLP** to indicate that the second slope value has been saved.

The system is now calibrated at three points and will return to normal operation.

18. Rinse the probe with deionized or distilled water.

4.3 Conductivity Calibration

IMPORTANT: System calibration is rarely required because of the factory calibration of the YSI Model 63. However, from time to time it is wise to check the system calibration and make adjustments when necessary.

Prior to calibration of the YSI Model 63, it is important to remember the following:

1. Always use clean, properly stored, NIST traceable calibration solutions (see *12 Accessories and Replacement Parts*). When filling a calibration container prior to performing the calibration procedures, make certain that the level of calibrant buffers is high enough in the container to cover the entire probe. Gently agitate the probe to remove any bubbles in the conductivity cell.
2. Rinse the probe with distilled water (and wipe dry) between changes of calibration solutions.
3. During calibration, allow the probe time to stabilize with regard to temperature (approximately 60 seconds) before proceeding with the calibration process. The readings after calibration are only as good as the calibration itself.
4. Perform conductivity calibration at a temperature as close to 25°C as possible. This will minimize any temperature compensation error.

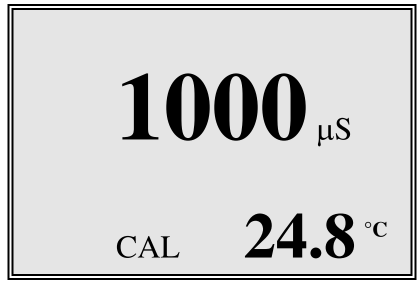
Follow these steps to perform an accurate calibration of the YSI Model 63:

1. Turn the instrument on and allow it to complete its self test procedure.
2. Select a calibration solution which is most similar to the sample you will be measuring.
    - For sea water choose a 50 mS/cm conductivity standard (YSI Catalog# 3169)
    - For fresh water choose a 1 mS/cm conductivity standard (YSI Catalog# 3167)
    - For brackish water choose a 10 mS/cm conductivity standard (YSI Catalog # 3168)
3. Place at least 7 inches of solution in the plastic container or a clean glass beaker.

NOTE: Do NOT use the 100 mL graduated cylinder. The diameter of the cylinder is too small for accurate conductivity measurements.

4. Use the **MODE** key to advance the instrument to display conductivity.
5. Insert the probe into the solution deep enough to completely cover the probe. Both conductivity ports must be submerged (see *Figure 6* on the following page).
6. Allow at least 60 seconds for the temperature reading to become stable.
7. Move the probe vigorously from side to side to dislodge any air bubbles from the electrodes.
8. Press and release the **UP ARROW** and **DOWN ARROW** keys at the same time.

The **CAL** symbol will appear at the bottom left of the display to indicate that the instrument is now in Calibration mode.



9. Use the **UP ARROW** or **DOWN ARROW** key to adjust the reading on the display until it matches the value of the calibration solution you are using.
10. Once the display reads the exact value of the calibration solution being used (the instrument will make the appropriate compensation for temperature variation from 25°C), press the **ENTER** key. The word "**SAVE"** will flash across the display for a second indicating that the calibration has been accepted.

The YSI Model 63 is designed to retain its last conductivity calibration permanently. Therefore, there is no need to calibrate the instrument after battery changes or power down.

4.4 Making Measurements

After the system has been set-up and pH has been calibrated as described in *4.2 pH Calibration*, it is ready to make measurements. Simply insert the probe into the sample, shake gently to remove any trapped air bubbles and wait for the readings to stabilize (approximately 60 seconds). The first pH reading after storage in buffers may take longer to stabilize (5 to 10 minutes), therefore, the probe should be stored in the transport chamber when making field measurements. It is important that the probe be inserted into the sample far enough so that the pH, temperature and conductivity sensors are covered by the liquid (see *Figure 6*).

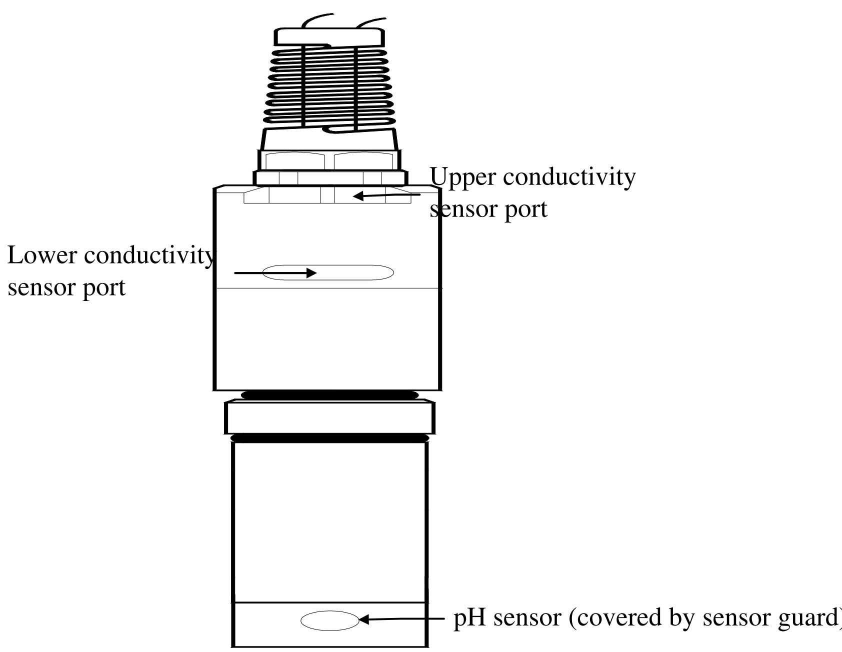
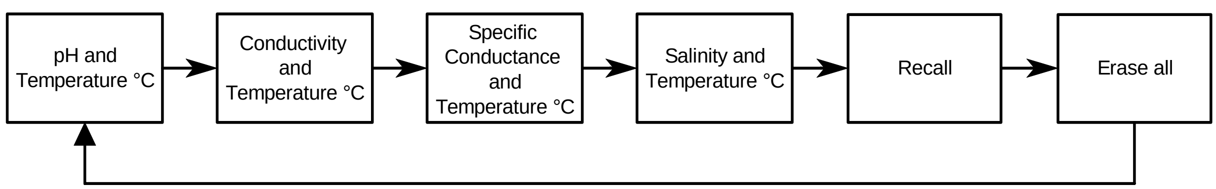


Figure 6

The Model 63 has six modes:

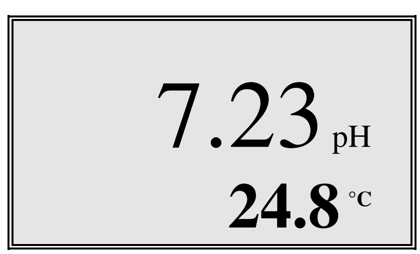
- **pH** -- Displays pH and temperature (°C).
- **Conductivity** -- A measurement of the conductive material in the liquid sample without regard to temperature. Also displays temperature (°C).
- **Specific Conductance** -- Also known as temperature compensated conductivity which automatically adjusts the reading to a calculated value which would have been read if the sample had been at 25° C (or some other reference temperature which you choose). See section *5 Advanced Conductivity Setup*. Also displays temperature (°C).
- **Salinity**-- A calculation done by the instrument electronics, based upon the conductivity and temperature readings. Also displays temperature (°C).
- **Recall** -- Allows previously stored data to be displayed.
- **Erase all** -- Allows ALL previously stored data to be deleted.

To change between the Model 63 modes, simply press and release the **MODE** key. The Model 63 will cycle through the modes as follows:

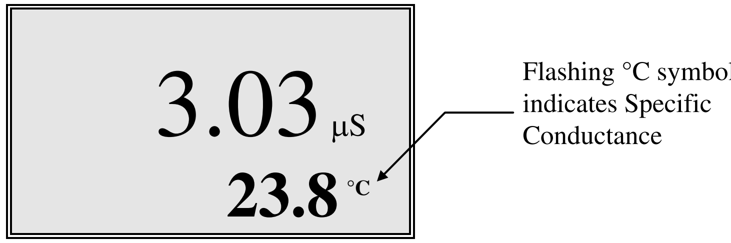


NOTE: When the Model 63 is turned off, it will "remember" which mode you used last and will return to that mode the next time it is turned on. If turned off while in recall or erase mode, it will default to pH mode when turned on.

To determine the current mode of the Model 63, carefully observe the small legends at the far right side of the LCD. If the instrument is reading **pH,** the large numbers on the display will be followed by **pH** as shown below.

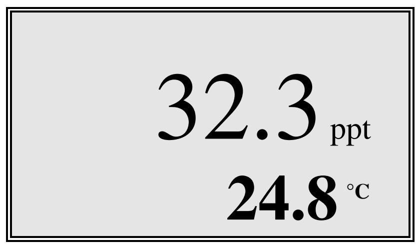


If the instrument is reading **Conductivity,** (not temperature compensated) the large numbers on the display will be followed by either a **µS** or an **mS.** Additionally, the small portion of the display will show the **° C** <u>**NOT**</u> flashing.



If the instrument is reading **Specific Conductance,** the large numbers on the display will be followed by either a **µS** or an **mS.** Additionally, the small portion of the display will show the **°C** flashing on and off.

If the instrument is reading **Salinity**, the large numbers on the display will be followed by a **ppt.**



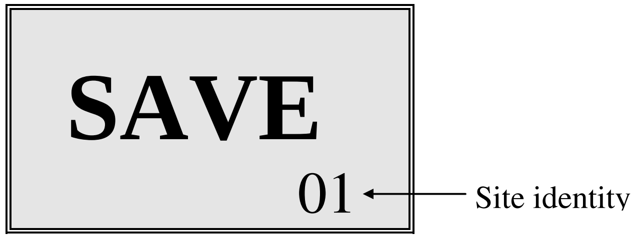
4.5 Autoranging & Range Searching

The YSI Model 63 is an autoranging instrument. This means that regardless of the conductivity or salinity of the solution (within the specifications of the instrument) all you need to do to get the most accurate reading is to put the probe in the sample. This feature makes the Model 63 as simple as possible to operate.

When you first place the Model 63 probe into a sample or calibration solution, and again when you first remove the probe the instrument will go into a range search mode that may take as long as 5 seconds. During some range searches the instrument display will flash **rANG** to indicate its movement from one range to another. The length of the range search depends on the number of ranges which must be searched in order to find the correct range for the sample. During the range search, the instrument will appear to freeze on a given reading for a few seconds then, once the range is located, will pinpoint the exact reading on the display. The display may also switch to **00.0** for a second or two during a range search before it selects the proper range.

4.6 Saving Data

The Model 63 is equipped with non-volatile memory that is capable of storing up to 50 different sets of readings. Non-volatile means that you do not need to worry that your data will be lost due to a power failure or interruption, such as when the batteries are removed. Each set consists of pH, conductivity, specific conductance, salinity and temperature. The Model 63 will also assign a site identity number to each set of readings to allow easy review of the data. This feature is useful in situations where transcribing data is difficult or not available.



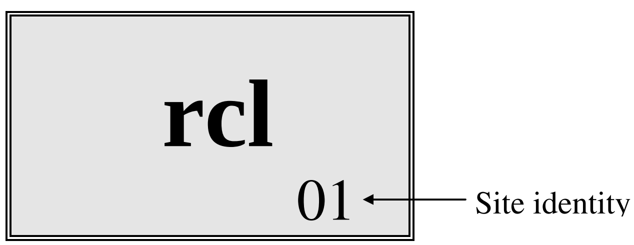
While pH, conductivity, specific conductance or salinity is displayed on the screen, press the **ENTER** key and hold it for approximately 2 seconds. The meter will flash **SAVE** on the display along with the current site identity (1 through 50) being used.

When all 50 sites are full, the display will flash **FULL** on the screen. This message will remain on the screen (even after power down) until a key is pushed.

Once you have acknowledged the memory is full, any subsequent saved data will begin overwriting existing data starting with site #1. No additional warning will be displayed.

4.7 Recalling Stored Data

1. To put the Model 63 into the **RECALL** mode, press the **MODE** key until **"rcl"** is displayed on the screen along with the site ID number in the lower right corner.



2. Press the **ENTER** key to review the last set of data that was saved. The Model 63 will display the pH and temperature. Another press of the **ENTER** key will display the conductivity and the temperature.
3. Depress the **ENTER** key again and again to review the specific conductance and salinity readings. All readings are displayed with the temperature.
4. Press the **UP ARROW** key to move up through the saved sets of data.
5. Press the **DOWN ARROW** key to move down through the saved sets of data.
6. When the correct Site ID# is displayed, press the **ENTER** key to display the data.

7. When you have finished recalling data, press **MODE** two times to return to normal operation.

NOTE: The Model 63 will recall data as a list. When the **UP ARROW** is pressed the Model 63 will display the Site ID# for the previously recorded data. For example: If you are reviewing Site ID# 5 and the **UP ARROW** is pressed, the Model 63 will display Site ID#4. If you are reviewing Site ID# 5 and Site ID# 5 was the last set of data stored, the **DOWN ARROW** key will display Site ID# 1.

Here is an example of the Model 63 memory.

Site ID #1
Site ID #2
Site ID #3 ←If the **UP ARROW** key was pressed the Model 63 would display Site ID #2
Site ID #4
Site ID #5

4.8 Erasing Stored Data

1. To erase the data that is stored in the Model 63's memory, press the **MODE** key until the Model 63 displays **ErAS** on the screen.
2. Press and hold the **DOWN ARROW** and **ENTER** keys simultaneously for approximately 5 seconds.

ErAS

3. Successful erasure is indicated by the Model 63 displaying **DONE** on the display for 1 to 2 seconds.

dOnE

The instrument will automatically change to pH mode after completion and the next saved data will be stored in site ID# 1.

IMPORTANT: Data in all 50 site ID's will be erased completely and will be lost forever. Do not use the erase function until all recorded data has been transcribed to an archive outside the Model 63.

4.9 Display Backlight

At times it may be necessary to take measurements with the Model 63 in dark or poorly lit areas. To help in this situation, the Model 63 comes equipped with a backlight which will illuminate the display so that it can be easily read. To activate the backlight, press and hold the **LIGHT** key. The display will remain lit as long as the key is pressed. When you release it, the light goes out to preserve battery life.

5. Advanced Conductivity Setup

The default settings of the YSI Model 63 are appropriate for the vast majority of measurement applications. However, some measurement applications require very specific measurement criteria. For that reason, we have made the YSI Model 63 flexible to accommodate these "advanced users."

If, for example, you are using the YSI Model 63 for a process control application which requires that the conductivity readings be compensated to 20°C instead of 25°C -- this is the chapter to read. Or, if your application for the YSI Model 63 involves the measurement of a very specific saline solution, the default temperature coefficient may need to be changed to get the very best measurement of that specific salt.

IMPORTANT: There is never a need to enter Advanced Setup Mode unless your special measurement application calls for a change in reference temperature and or temperature coefficient. Therefore, unless you are certain that your application requires a change to one or both of these criteria, do not modify the default reference temperature (25°C) or the default temperature coefficient (1.91%).

NOTE: Changing the reference temperature or temperature coefficient does not affect salinity readings which are always referenced to seawater at 15°C. See *9.3 Salinity* for details.

5.1 Changing The Temperature Coefficient

Follow these steps to modify the temperature coefficient of the Model 63.

1. Turn the instrument on and wait for it to complete its self test procedure.
2. Use the **MODE** key to advance the instrument to display conductivity.
3. Press and release both the **DOWN ARROW** and the **MODE** keys at the same time.

The **CAL** symbol will appear at the bottom left of the display. The large portion of the display will show **1.91 %** (or a value set previously using Advanced Setup).

4. Use the **UP ARROW** or **DOWN ARROW** key to change the value to the desired new temperature coefficient.
5. Press the **ENTER** key. The word "**SAVE**" will flash across the display for a second to indicate that your change has been accepted.
6. Press the **MODE** key to return to normal operation; the CAL symbol will disappear from the display.

5.2 Changing The Reference Temperature

Follow these steps to modify the reference temperature of the Model 63.

1. Turn the instrument on and wait for it to complete its self test procedure.
2. Use the **MODE** key to advance the instrument to display conductivity.
3. Press and release both the **DOWN ARROW** and the **MODE** keys at the same time.

The **CAL** symbol will appear at the bottom left of the display. The large portion of the display will show **1.91 %** (or a value set previously using Advanced Setup).

4. Press and release the **MODE** key; the large portion of the display will show **25.0C** (or a value set previously using Advanced Setup).
5. Use the **UP ARROW** or **DOWN ARROW** key to change the value to the desired new reference temperature (any value between 15°C and 25°C is acceptable).
6. Press the **ENTER** key. The word "**SAVE**" will flash across the display for a second to indicate that your change has been accepted.
7. The instrument will automatically return to normal operation mode.

5.3 Changing Conductivity From Autoranging To Manual Ranging

If your application is easier to perform using a manual range which you select, the YSI Model 63 allows you to turn off the default autoranging feature. While you are making conductivity or temperature compensated conductivity measurements, simply press and release the **UP ARROW** key. Each additional press of the **UP ARROW** key will cycle the Model 63 to a different manual range until you return again to autoranging. Five pushes of the **UP ARROW** key will cycle the Model 63 through the four manual ranges and return the instrument to autoranging.

NOTE: You may see an error message in some manual ranges if the manual range selected is not adequate for the sample you are measuring. If this happens, simply press and release the **UP ARROW** key again until a range is selected which is suitable for your sample. If you get lost and don't know if you're in a manual range or autoranging, simply turn the instrument off and back on. Also note that the conductivity units will flash while you are in manual range. The instrument will always default to autoranging when first turned on.

The four conductivity ranges of the YSI Model 63 are:

Range 1	Range 2	Range 3	Range 4
0 to 499.9 µS/cm	0 to 4999 µS/cm	0 to 49.99 mS/cm	0 to 200.0 mS/cm

6. Maintenance

6.1 pH Sensor Precautions

1. When making measurements or performing the calibration procedure, make certain that the level of sample or pH buffer is high enough to cover both the pH and temperature sensors.
2. Rinse the probe with deionized water between changes of calibration buffer solutions.
3. During pH calibration, allow the sensors time to stabilize with regard to temperature (approximately 60 seconds) before proceeding with the calibration protocol. The pH readings after calibration are only as good as the calibration itself.
4. Clean and store the probe according to the instructions found below.

6.2 pH Sensor Cleaning

Cleaning is required whenever deposits or contaminants appear on the glass pH sensor. Unscrew and remove the small guard that protects the pH sensor. Use tap water and a clean cloth or lens cleaning tissue to remove all foreign material from the glass sensor.

If good pH response is not restored by the above procedure, perform the following additional procedure:

1. Soak the probe for 10 to 15 minutes in clean water containing a few drops of commercial dishwashing liquid.
2. GENTLY clean the glass bulb by rubbing with a cotton swab soaked in the cleaning solution.
3. Rinse the probe in clean water, wipe with a cotton swab saturated with clean water, and then rerinse with clean water.

If good pH response is still not restored by the above procedure, perform the following additional procedure:

1. Soak the pH sensor for 5 minutes in one molar (1 M) hydrochloric acid (HCl).
2. GENTLY clean the glass bulb by rubbing with a cotton swab soaked in the acid.
3. Rinse the probe in clean water, wipe with a cotton swab saturated with clean water, and then rerinse with clean water.
4. Reinstall the small guard that protects the pH sensor.

If biological contamination of the reference junction is suspected or if good response is not restored by the above procedures, perform the following additional cleaning step:

1. Soak the probe for approximately 1 hour in a 1 to 1 dilution of commercially-available chlorine bleach.
2. Rinse the probe with clean water and then soak for 1 hour in clean water to remove residual bleach from the junction.

6.3 pH Sensor Storage

For short term storage between measurements in the field (up to one week), place the probe in the transport chamber in the side of the instrument case. Make sure that the sponge inside the chamber is wet (tap water).

For long term storage (over one week), place the probe in the storage bottle (provided) containing a mixture of 50% pH 4 buffer and 50% 1.5M KCl. This will assure the fastest possible pH response. If this mixture is not available, storage in tap water is the next best choice. **Do NOT store the probe dry or in distilled or deionized water**.

NOTE: After storage in the pH 4/KCl solution described above, place the probe in the transport chamber in the side of the instrument case or soak the probe in pH 7 buffer for 5 to 10 minutes allowing it to acclimate before calibrating.

If the probe has been inadvertently left in air and the reference electrode junction has dried out, good function can usually be restored by soaking the probe in the pH 4/KCl solution described above.

6.4 Conductivity Sensor Cleaning

The single most important requirement for accurate and reproducible results in conductivity measurement is a clean cell. A dirty cell will change the conductivity of a solution by contaminating it.

NOTE: Always rinse the conductivity cell with clean water after each use.

To clean the conductivity cell:

1. Dip the cell in cleaning solution and agitate for two to three minutes. Any one of the foaming acid tile cleaners, such as Dow Chemical Bathroom Cleaner, will clean the cell adequately. When a stronger cleaning preparation is required, use a solution of 1:1 isopropyl alcohol and one molar (1 M) hydrochloric acid (HCl). Remove the cell from the cleaning solution.

2. Use the nylon brush (supplied) to dislodge any contaminants from inside the electrode chamber.

3. Repeat steps one and two until the cell is completely clean. Rinse the cell thoroughly in deionized, or clean tap water.

7. Discussion of Measurement Errors

7.1 pH Errors

There are two basic types of pH errors. The first type are errors related to limitations of instrument design and tolerances of components. The second type are errors due to basic sensor accuracy tolerances, mainly background signal, linearity, and variations in temperature coefficient. It is unlikely that the actual error in any measurement will be the maximum possible error.

Errors

- Component and circuitry error: ±0.03 pH
- pH error caused by sensor accuracy and temperature compensation:

  ±0.1 pH for measurements at 10°C from calibration temperature
  ±0.2 pH for measurements at 20°C from calibration temperature

7.2 Conductivity Errors

System accuracy for conductivity measurements is equal to the sum of the errors contributed by the environment and the various components of the measurement setup. These include:

- Instrument accuracy
- Cell-constant error
- Solution temperature offset
- Cell contamination (including air bubbles)
- Electrical noise
- Galvanic effects

Only the first three are of major concern for typical measurements, although the user should also be careful to see that cells are clean and maintained in good condition at all times.

Instrument Accuracy = ± .5% maximum
The accuracy specified for the range being used is the worst case instrument error.

Cell-Constant Error = ± .5% maximum
Although YSI cells are warranted to be accurate to within one percent, you should still determine the exact cell constant of your particular cell. Contamination or physical damage to the cell can alter the cell constant. Performing a calibration will eliminate any error which might arise because of cell constant change.

YSI cells are calibrated to within one percent of the stated cell constant at a single point. We consider these products to be usefully linear over most instrument ranges. The cell constant can be calibrated to ±0.35% accuracy with YSI conductivity calibrator solutions.

Temperature Error = ± 1% maximum
The solution temperature error is the product of the temperature coefficient and the temperature offset from 25°C, expressed as a percentage of the reading that would have been obtained at 25°C. The error is not necessarily a linear function of temperature. The statement of error is derived from a 25°C temperature offset and a 3%/°C temperature coefficient.

Total Error
Considering only the above three factors, system accuracy under worst case conditions will be ±2%, although the actual error will be considerably less if recommended and properly calibrated cells and instrument ranges are used. Additional errors, which can essentially be eliminated with proper handling, are described below.

Cell Contamination
This error is usually due to contamination of the solution being measured, which occurs when solution is carried-over from the last solution measured. Thus, the instrument might be correctly reporting the conductivity seen, but the reading does not accurately represent the value of the bulk solution. Errors will be most serious when low conductivity solutions are contaminated by carry-over from high conductivity solutions, and can then be of an order of magnitude or more.

Follow the cleaning instructions carefully before attempting low conductivity measurements with a cell of unknown history or one that has been previously used in higher value solutions.

An entirely different form of contamination sometimes occurs due to a buildup of foreign material directly on cell electrodes. While rare, such deposits have, on occasion, markedly reduced the effectiveness of the electrodes. The result is an erroneously low conductance reading.

Electrical-Noise Errors
Electrical noise can be a problem in any measurement range, but will contribute the most error and be the most difficult to eliminate when operating in the lowest ranges. The noise may be either line-conducted or radiated or both, and may require, grounding, shielding, or both.

Galvanic and Miscellaneous Effects
In addition to the error sources described above, there is another class of contributors that can be ignored for all but the most meticulous of laboratory measurements. These errors are always small and are generally completely masked by the error budget for cell-constant calibration, instrument accuracy, etc. Examples range from parasitic reactances associated with the solution container and its proximity to external objects to the minor galvanic effects resulting from oxide formation or deposition on electrodes. Only trial and error in the actual measurement environment can be suggested as an approach to reduce such errors. If the reading does not change as the setup is adjusted, errors due to such factors can be considered too small to see.

8. Troubleshooting

Error Messages

The instrument performs a Power On Self Test each time it is turned on. The following error messages are provided to facilitate troubleshooting. They appear on the LCD when an error is detected.

Symptom	Possible Cause	Action
1. Instrument will not turn on	• Low battery voltage • Batteries installed wrong • Meter requires service	• Replace batteries (pg 2) • Check battery polarity (pg 2) • Return system for service (pg 29)
2. Instrument "locks up"	• Instrument has received a shock • Batteries are low or damaged • System requires service	• Remove battery lid, wait 15 seconds for reset, replace lid. (pg 2) • Replace batteries (pg 2) • Return system for service (pg 29)
3. Conductivity will not calibrate	• Conductivity standards out of spec. • Conductivity cell is contaminated	• Recalibrate with known good standards (pg 11) • Clean conductivity cell (pg 20)
4. pH will not calibrate due to unstable readings (decimal point keeps flashing)	• pH sensor is fouled • pH sensor is bad • System requires service	• Clean pH sensor (pg 20) • Replace pH sensor (pg 4, 33) • Return system for service (pg 29)
5. pH readings are inaccurate	• Calibration is required • pH calibration buffers out of spec • Calibration procedure not correct • Sample temperature is over 20°C from calibration temperature • pH sensor is fouled or damaged • pH Sensor is bad • System requires service	• Recalibrate with known good standards (pg 6) • Calibrate within ±20°C of sample temp (±10°C for best results) • Clean pH sensor (pg 20) • Replace pH sensor (pg 4, 33) • Return system for service (pg 29)
6. Conductivity readings are inaccurate	• Cell is contaminated • Calibration is required • Temperature coefficient is set incorrectly • Reference temperature is set incorrectly • Readings are or are not temperature compensated.	• Clean conductivity cell (pg 21) • See *Conductivity Calibration* (pg 11) • See Changing The Temperature Coefficient (pg 18) • See *Changing The Reference Temperature* (pg 19) • See *Making Measurements* (pg 12)
7. LCD displays "LO BAT"	• Batteries are low or damaged • System requires service	• Replace batteries (pg 2) • Return system for service (pg 29)
8. Main Display reads “nOnE”	• During recall, no data is currently stored in memory.	• Store data before attempting to recall (pg 14)
9. pH Display reads “OVEr”	• When calibrating: pH level is over range allowed for the buffer value selected. • When measuring, pH level is > 14	• Recalibrate with known good standards (pg 6) • Clean pH sensor (pg 20) • Replace pH sensor (pg 4, 33) • Return system for service (pg 29)

Symptom	Possible Cause	Action
10. Conductivity/Salinity Display reads “OVEr”	• When calibrating: User cell constant cal K is >5.25 • When measuring: Conductivity reading is >200 mS • Salinity reading is >80 ppt	• Recalibrate with known good standards (pg 11) • Clean conductivity cell (pg 21) • Return system for service (pg 29)
11. pH Display reads “undr”	• When calibrating, pH level is under range allowed for the buffer value selected. • When measuring, pH level is < 0	• Recalibrate with known good standards (pg 6) • Clean pH sensor (pg 20) • Replace pH sensor (pg 4, 33) • Return system for service (pg 29)
12. Conductivity Display reads “undr”	• User cell constant cal K is <4.9	• Recalibrate with known good standards (pg 11) • Clean conductivity cell (pg 21) • Return system for service (pg 29)
13. Main Display reads “OVEr” (Secondary display reads “ovr”)	• Temperature reading is >75°C	• Measure samples at a temperature within the range of the system.
14. Main Display reads “undr” (Secondary display reads “udr”)	• Temperature reading is <-5°C	• Measure samples at a temperature within the range of the system.
15. Main display reads “PErr”	• User cell constant cal K is 0.0 • Incorrect sequence of keystrokes.	• See “Advanced Setup” chapter (pg 18) • Refer to manual section for step by step instruction for the function you are attempting.
16. Main display reads “LErr”	• In temperature compensated conductivity mode, temperature exceeds the values computed using user defined temperature coefficient and/or reference temperature. • In cell constant cal mode, temperature exceeds the values computed using user defined temperature coefficient and/or reference temperature.	• Adjust user defined temperature coefficient or reference temperature. (pg 18)
17. Main display reads “Err” (Secondary display reads “ra”)	• System has failed its RAM test check procedure.	• Turn instrument OFF and back ON again. • Return the system for service (pg 29)
18. Main display reads “Err” (Secondary display reads “ro”)	• System has failed its ROM test check procedure.	• Turn instrument OFF and back ON again. • Return the system for service (pg 29)
19. Main display reads “FAIL” (Secondary display reads “eep”)	• EEPROM has failed to respond in time.	• Return the system for service (pg 29)
20. Readings on main display don’t change	• Meter is in recall mode.	• Press MODE key to return to Normal Operation (pg 5, 12)

9. Principles of Operation

9.1 pH

The YSI Model 63 employs a field replaceable pH sensor for the determination of hydrogen ion concentration. The sensor is a combination electrode consisting of a proton selective glass reservoir filled with buffer at approximately pH 7 and a Ag/AgCl reference electrode which utilizes gelled electrolyte. A silver wire coated with AgCl is immersed in the buffer reservoir. Protons (H+ ions) on both sides of the glass (media and buffer reservoir) selectively interact with the glass, setting up a potential gradient across the glass membrane. Since the hydrogen ion concentration in the internal buffer solution is invariant, this potential difference, determined relative to the Ag/AgCl reference electrode, is proportional to the pH of the media.

Our testing of the Model 63 pH sensor indicates that it should provide long life, good response time and accurate readings in most environmental waters, including fresh water of low ionic strength. No special sensor is required (nor offered) for water of low conductivity.

pH Calibration And Effect Of Temperature

The software of the YSI Model 63 calculates pH from the established linear relationship between pH and the millivolt output as defined by a variation of the Nernst equation:

$$E = E_o + \frac{2.3RT}{nF} * pH$$

where E = millivolts output
E_o = a constant associated with the reference electrode
T = temperature of measurement in degrees Kelvin
R, n, and F are invariant constants

Thus, in simplified ***y = mx + b*** form, it is (mv output) = (slope)x(pH) + (intercept). In order to quantify this simple relationship, the instrument must be calibrated properly using buffers of known pH values. In this procedure, the millivolt values for two standard buffer solutions are experimentally established and used by the YSI Model 63 software to calculate the slope and intercept of the plot of millivolts vs. pH. Once this calibration procedure has been carried out, the millivolt output of the probe in any media can readily be converted by the YSI Model 63 software into a pH value, *as long as the calibration and the reading are carried out at the same temperature.* This last qualifier is almost never met in actual environmental measurements, thus, a mechanism must be in place to compensate for temperature or, in other words, to accurately convert the slope and intercept of the plot of pH vs. millivolts established at T_c (temperature of calibration) into a slope and intercept at T_m (temperature of measurement). Fortunately, the Nernst equation provides a basis for this conversion.

According to the Nernst equation as shown above, the slope of the plot of pH vs. millivolts is *directly proportional* to the absolute temperature in degrees Kelvin. Thus, if the slope of the plot is experimentally determined to be 59 mv/pH unit at 298 K (25 C), then the slope of the plot at 313 K (40 C) must be (313/298) * 59 = 62 mv/pH unit. At 283 K (10 C), the slope is calculated to be 56 mv/pH unit ((283/298) * 59). Determination of the slope of pH vs. mv plots at temperatures different from T_c is thus relatively simple. In order to establish the intercept of the new plot, the point where plots of pH vs. mv at different temperatures intersect (the isopotential point) must be known. Using standard pH determination protocol, the YSI Model 63 software assigns the isopotential point as the mv reading at pH 7 and then calculates the intercept using this assumption. Once the slope and intercept to the plot of pH vs. mv are assigned at the new temperature, the calculation of pH under the new temperature conditions is straightforward, and is automatically carried out by the software.

Number of pH Calibration Points

When calibrating the YSI Model 63, you have the choice of 1-point 2-point, or 3-point calibration. **Perform a 2 or 3 point calibration at least once per day for accurate results.**

Select the **1-point** option only if you are adjusting a previous calibration. If a 2-point or 3-point calibration has been performed previously (at least once per day), you can adjust the calibration by carrying out a 1-point calibration at pH 7 (or pH 6.86). This calibration procedure adjusts only the pH offset and leaves the previously-determined slope unaltered.

Select the **2-point** option to calibrate the pH probe using only two calibration standards. In this procedure, the pH sensor is calibrated using a pH 7 (or pH 6.86) buffer and *one additional* buffer. A two point calibration procedure (as opposed to a 3-point procedure) can save time if the pH of the sample is known to be either basic or acidic. For example, if the pH of a sample is known to vary between 5.5 and 7, a two point calibration with pH 7 and pH 4 buffers is appropriate. Three point calibration with an additional pH 10 buffer will not increase the accuracy of this measurement since the pH is not within this higher range.

Select the **3-point** option to calibrate the pH probe using three calibration solutions. In this procedure, the pH sensor is calibrated with a pH 7 (or pH 6.86) buffer and two additional buffers. The 3-point calibration method assures maximum accuracy when the pH of the media to be monitored cannot be anticipated.

9.2 Conductivity

The conductivity cell utilizes four pure nickel electrodes for the measurement of solution conductance. Two of the electrodes are current driven, and two are used to measure the voltage drop. The measured voltage drop is then converted into a conductance value in milli-Siemens (millimhos). To convert this value to a conductivity (specific conductance) value in milli-Siemens per cm (mS/cm), the conductance is multiplied by the cell constant which has units of reciprocal cm (cm^{-1}). The cell constant for the Model 63 conductivity cell is 5.0/cm $\pm$ 4%. For most applications, the cell constant is automatically determined (or confirmed) with each deployment of the system when the calibration procedure is followed. Solutions with conductivity's of 1.00, 10.0, 50.0, and 100.0 mS/cm, which have been prepared in accordance with recommendation 56-1981 of the Organisation Internationale de Métrologie Légale (OIML) are available from YSI. The instrument output is in µS/cm or mS/cm for both conductivity and specific conductance. The multiplication of cell constant times conductance is carried out automatically by the software.

Temperature Effect On Conductivity

The conductivity of solutions of ionic species is highly dependent on temperature, varying as much as 3% for each change of one degree Celsius (temperature coefficient = 3%/C). In addition, the temperature coefficient itself varies with the nature of the ionic species present.

Because the exact composition of a natural media is usually not known, it is best to report a conductivity at a particular temperature, e.g. 20.2 mS/cm at 14 C. However, in many cases, it is also useful to compensate for the temperature dependence in order to determine at a glance if gross changes are occurring in the ionic content of the medium over time. For this reason, the Model 63 software also allows the user to output conductivity data in either raw or temperature compensated form. If "Conductivity" is selected, values of conductivity which are **NOT** compensated for temperature are output to the display. If "Specific Conductance" is selected, the Model 63 uses the temperature and raw conductivity values associated with each determination to generate a specific conductance value compensated to a user selected reference temperature (see *Advanced Setup*) between 15 C and 25 C. Additionally the user can select any temperature coefficient from 0% to 4% (see *Advanced Setup).* Using the Model 63 default reference temperature and temperature coefficient (25 C and 1.91%), the calculation is carried out as in the equation below:

$$\text{Specific Conductance } (25°C) = \frac{\text{Conductivity}}{1 + TC * (T - 25)}$$

As noted above, unless the solution being measured consists of pure KCl in water, this temperature compensated value will be somewhat inaccurate, but the equation with a value of TC = 0.0191 will provide a close approximation for solutions of many common salts such as NaCl and NH_4Cl and for seawater.

9.3 Salinity

Salinity is determined automatically from the Model 63 conductivity and temperature readings according to algorithms found in *Standard Methods for the Examination of Water and Wastewater (ed. 1995).* The use of the Practical Salinity Scale 1978 results in values which are unitless, since the measurements are carried out in reference to the conductivity of standard seawater at 15°C. However, the unitless salinity values are very close to those determined by the previously-used method where the mass of dissolved salts in a given mass of water (parts per thousand) was reported. Hence, the designation "ppt" is reported by the instrument to provide a more conventional output.

For further information on conductivity and the above standard information, refer to the ASTM document, *Standard Methods of Test for Electrical Conductivity of Water and Industrial Wastewater*, ASTM Designation D1125-82, and OIML *Recommendation Number 56.* ASTM symbols for conductivity, cell constant, and path length differ from those preferred in the general literature and also from those used in this manual.

9.4 Temperature

The YSI Model 63 system utilizes a thermistor which changes predictably in resistance with temperature variation. The algorithm for conversion of resistance to temperature is built-in to the Model 63 software, and accurate temperature readings, in degrees Celsius, are provided automatically. No calibration or maintenance of the temperature sensor is required.

10. Warranty and Repair

YSI Model 63 Meters are warranted for two years from date of purchase by the end user against defects in materials and workmanship. YSI Model 63 probes, cables and sensors are warranted for one year from date of purchase by the end user against defects in material and workmanship. Breakage of pH sensors is NOT covered under warranty. Within the warranty period, YSI will repair or replace, at its sole discretion, free of charge, any product that YSI determines to be covered by this warranty.

To exercise this warranty, write or call your local YSI representative, or contact YSI Customer Service in Yellow Springs, Ohio. Send the product and proof of purchase, transportation prepaid, to the Authorized Service Center selected by YSI. Repair or replacement will be made and the product returned, transportation prepaid. Repaired or replaced products are warranted for the balance of the original warranty period, or at least 90 days from date of repair or replacement.

Limitation of Warranty

This Warranty does not apply to any YSI product damage or failure caused by (i) failure to install, operate or use the product in accordance with YSI's written instructions, (ii) abuse or misuse of the product, (iii) failure to maintain the product in accordance with YSI's written instructions or standard industry procedure, (iv) any improper repairs to the product, (v) use by you of defective or improper components or parts in servicing or repairing the product, or (vi) modification of the product in any way not expressly authorized by YSI.

THIS WARRANTY IS IN LIEU OF ALL OTHER WARRANTIES, EXPRESSED OR IMPLIED, INCLUDING ANY WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. YSI's LIABILITY UNDER THIS WARRANTY IS LIMITED TO REPAIR OR REPLACEMENT OF THE PRODUCT, AND THIS SHALL BE YOUR SOLE AND EXCLUSIVE REMEDY FOR ANY DEFECTIVE PRODUCT COVERED BY THIS WARRANTY. IN NO EVENT SHALL YSI BE LIABLE FOR ANY SPECIAL, INDIRECT, INCIDENTAL OR CONSEQUENTIAL DAMAGES RESULTING FROM ANY DEFECTIVE PRODUCT COVERED BY THIS WARRANTY.

YSI Authorized Service Centers

Please visit www.ysi.com or contact YSI Technical Support for the nearest authorized service center.

YSI Incorporated • Technical Support • Phone: +1 937 767-7241 • 800 897-4151 • Fax: 937 767-1058 • Email: environmental@ysi.com

10.1 Cleaning Instructions

NOTE: Before they can be serviced, equipment exposed to biological, radioactive, or toxic materials must be cleaned and disinfected. Biological contamination is presumed for any instrument, probe, or other device that has been used with body fluids or tissues, or with waste water. Radioactive contamination is presumed for any instrument, probe or other device that has been used near any radioactive source.

If an instrument, probe, or other part is returned or presented for service without a Cleaning Certificate, and if in our opinion it represents a potential biological or radioactive hazard, our service personnel reserve the right to withhold service until appropriate cleaning, decontamination, and certification has been completed. We will contact the sender for instructions as to the disposition of the equipment. Disposition costs will be the responsibility of the sender.

When service is required, either at the user's facility or at YSI, the following steps must be taken to insure the safety of our service personnel.

1. In a manner appropriate to each device, decontaminate all exposed surfaces, including any containers. 70% isopropyl alcohol or a solution of 1/4 cup bleach to 1 gallon tap water are suitable for most disinfecting. Instruments used with waste water may be disinfected with .5% Lysol if this is more convenient to the user.

2. The user shall take normal precautions to prevent radioactive contamination and must use appropriate decontamination procedures should exposure occur.

3. If exposure has occurred, the customer must certify that decontamination has been accomplished and that no radioactivity is detectable by survey equipment.

4. Any product being returned to the YSI Repair Center, should be packed securely to prevent damage.

5. Cleaning must be completed and certified on any product before returning it to YSI.

10.2 Packing Instructions

1. Clean and decontaminate items to insure the safety of the handler.
2. Complete and include the Cleaning Certificate.
3. Place the product in a plastic bag to keep out dirt and packing material.
4. Use a large carton, preferably the original, and surround the product completely with packing material.
5. Insure for the replacement value of the product.

Cleaning Certificate

Organization ________________________________

Department ________________________________

Address ____________________________________

City _______________ State ______ Zip __________

Country __________________ Phone ____________

Model No. of Device ______ Lot Number __________

Contaminant (if known) _________________________

______

Cleaning Agent(s) used _________________________

Radioactive Decontamination Certified?

(Answer only if there has been radioactive exposure)

___ Yes ___ No

Cleaning Certified By __________________________

Name Date

11. Required Notice

The Federal Communications Commission defines this product as a computing device and requires the following notice:

This equipment generates and uses radio frequency energy and if not installed and used properly, may cause interference to radio and television reception. There is no guarantee that interference will not occur in a particular installation. If this equipment does cause interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try to correct the interference by one or more of the following measures:

- re-orient the receiving antenna
- relocate the computer with respect to the receiver
- move the computer away from the receiver
- plug the computer into a different outlet so that the computer and receiver are on different branch circuits.

If necessary, the user should consult the dealer or an experienced radio/television technician for additional suggestions. The user may find the following booklet, prepared by the Federal Communications Commission, helpful: "How to Identify and Resolve Radio-TV Interference Problems." This booklet is available from the U.S. Government Printing Office, Washington, DC 20402, Stock No. 0004-000-00345-4.

12. Accessories and Replacement Parts

The following parts and accessories are available from YSI or any Franchise Dealer authorized by YSI.

YSI Order Number	Description
3161	Conductivity Calibration Solution 1,000 µ/cm (1 Quart)
3163	Conductivity Calibration Solution 10,000 µ/cm (1 Quart)
3165	Conductivity Calibration Solution 100,000 µ/cm (1 Quart)
3167	Conductivity Calibration Solution 1,000 µ/cm (8 pints)
3168	Conductivity Calibration Solution 10,000 µ/cm (8 pints)
3169	Conductivity Calibration Solution 50,000 µ/cm (8 pints)
3821	pH Buffer Solution, 4
3822	pH Buffer Solution, 7
3823	pH Buffer Solution, 10
5050	Carrying Case
031133	pH sensor
113165	Conductivity Probe/Cable Assembly (10 feet)
113166	Conductivity Probe/Cable Assembly (25 feet)
113157	Conductivity Probe/Cable Assembly (50 feet)
113158	Conductivity Probe/Cable Assembly (100 feet)
031163	Front Case Cover
055242	Rear Case Cover
055210	Battery Cover Kit
055204	Case Gasket and Screw
031129	Main Board Assembly
038213	Electrode Cleaning Brush, Conductivity
031189	Graduated Cylinder, 100 mL
060992	Container, Plastic (uses 060991 cap)
060991	Cap, Plastic Container (for 060992 container)

13. Appendix A - Specifications

Materials: ABS, Stainless Steel, and other materials

Dimensions:

Height:	9.5 inches	(24.13 cm)
Thickness:	2.2 inches	(5.6 cm)
Width:	3.5 inches max.	(8.89 cm)
Weight:	1.7 pounds (w/ 10' cable)	(.77 kg)
Display:	2.3"W x 1.5"L	(5.8 cm W x 3.8 cm L)

Power: 6 AA-size Alkaline Batteries (included)

Approximately 100 hours operation from each new set of batteries

Automatic shutoff after 10 hours without a key press

Water Tightness: Meets or exceeds IP65 standards

Probe Operating Environment

Medium: fresh, sea, or polluted water and most other liquid solutions.

Temperature: -5 to +75 °C

Depth: 0 to 10, 0 to 25, 0 to 50, or 0 to 100 feet (depending on cable length)

Meter Ambient Operating/Storage Temperature: -5 to +45 °C

System Performance Specifications

Measurement	Range	Resolution	Accuracy
pH	0 to 14	0.01 unit	± 0.1 pH unit within ±10°C of calibration temperature **or** ± 0.2 pH unit within ±20°C of calibration temperature
Conductivity	0 to 499.9 µS/cm 0 to 4999 µS/cm 0 to 49.99 mS/cm 0 to 200.0 mS/cm	0.1 µS/cm 1.0 µS/cm 0.01 mS/cm 0.1 mS/cm	± 0.5% FS
Salinity	0 to 80 ppt	0.1 ppt	± 2%, or ± 0.1 ppt
Temperature	-5 to +75 °C	0.1 °C	± 0.1°C ±1 LSD

Adjustable Conductivity Reference Temperature: 15°C to 25°C

Adjustable Temperature Compensation Factor for Conductivity: 0% to 4%

pH Response Time: 3 sec for 95% of the change at 25°C

Temperature Response Time: 20 sec for 95% of the change

Temperature Compensation: Automatic

Range: User selected or Autoranging for Conductivity

Data Storage: 50 points with ID number.

14. Appendix B - Health and Safety

YSI pH 4, 7 & 10 Buffer Solutions: 3821, 3822, 3823

pH 4 Ingredients:

- ☞ Potassium Hydrogen Phthalate
- ☞ Formaldehyde
- ☞ Water

pH 7 Ingredients:

- ☞ Sodium Phosphate, Dibasic
- ☞ Potassium Phosphate, Monobasic
- ☞ Water

pH 10 Ingredients:

- ☞ Potassium Borate, Tetra
- ☞ Potassium Carbonate
- ☞ Potassium Hydroxide
- ☞ Sodium (di) Ethylenediamine Tetraacetate
- ☞ Water

CAUTION - Avoid inhalation, skin contact, eye contact or ingestion. May affect mucous membranes.

Inhalation may cause severe irritation and be harmful. Skin contact may cause irritation; prolonged or repeated exposure may cause Dermatitis. Eye contact may cause irritation or conjunctivitis. Ingestion may cause nausea, vomiting and diarrhea.

FIRST AID:

INHALATION - Remove victim from exposure area to fresh air immediately. If breathing has stopped, give artificial respiration. Keep victim warm and at rest. Seek medical attention immediately.

SKIN CONTACT - Remove contaminated clothing immediately. Wash affected area with soap or mild detergent and large amounts of water (approx. 15-20 minutes). Seek medical attention immediately.

EYE CONTACT - Wash eyes immediately with large amounts of water (approx. 15-20 minutes), occasionally lifting upper and lower lids. Seek medical attention immediately.

INGESTION - If victim is conscious, immediately give 2 to 4 glasses of water and induce vomiting by touching finger to back of throat. Seek medical attention immediately.

15. Appendix C - pH Buffer Values

YSI pH 4, 7 and 10 Buffer Solutions: 3821, 3822, 3823

The following table lists the values of YSI pH buffer solutions at various temperatures.

Temperature	pH 4	pH 7	pH 10
0°C	4.01	7.13	10.34
5°C	4.00	7.10	10.26
10°C	4.00	7.07	10.19
15°C	4.00	7.05	10.12
20°C	4.00	7.02	10.06
25°C	4.01	7.00	10.00
30°C	4.01	6.99	9.94
35°C	4.02	6.98	9.90
40°C	4.03	6.97	9.85
50°C	4.06	6.97	9.78
60°C	4.09	6.98	9.70

NIST pH 4.01, 6.86 and 9.18 Buffers: SRM 185g, SRM 186-Ie/IIe, SRM 187c

The following table lists the values of NIST pH buffer solutions at various temperatures.

Temperature	pH 4.01	pH 6.86	pH 9.18
0°C	4.005	6.984	9.463
5°C	4.003	6.950	9.395
10°C	4.001	6.924	9.333
15°C	4.002	6.899	9.277
20°C	4.003	6.879	9.226
25°C	4.005	6.863	9.180
30°C	4.010	6.852	9.139
35°C	4.020	6.844	9.102
37°C	4.025	6.842	N/A
40°C	4.033	6.840	9.070
45°C	4.047	6.837	9.042
50°C	4.061	6.836	9.018

16. Appendix D - Temperature Correction Data

Conductivity Temperature Correction Data for Typical Solutions

A. Potassium Chloride** (KCl)

Concentration: 1 mole/liter			Concentration: 1 x 10^{-1} mole/liter		
°C	mS/cm	%/°C (to 25°C)	°C	mS/cm	%/°C (to 25°C)
0	65.10	1.67	0	7.13	1.78
5	73.89	1.70	5	8.22	1.80
10	82.97	1.72	10	9.34	1.83
15	92.33	1.75	15	10.48	1.85
20	101.97	1.77	20	11.65	1.88
25	111.90	1.80	25	12.86	1.90
			30	14.10	1.93
			35	15.38	1.96
			37.5	16.04	1.98
			40	16.70	1.99
			45	18.05	2.02
			50	19.43	2.04

Concentration: 1 x 10^{-2} mole/liter			Concentration: 1 x 10^{-3} mole/liter		
°C	mS/cm	%/°C (to 25°C)	°C	mS/cm	%/°C (to 25°C)
0	0.773	1.81	0	0.080	1.84
5	0.892	1.84	5	0.092	1.88
10	1.015	1.87	10	0.105	1.92
15	1.143	1.90	15	0.119	1.96
20	1.275	1.93	20	0.133	1.99
25	1.412	1.96	25	0.147	2.02
30	1.553	1.99	30	0.162	2.05
35	1.697	2.02	35	0.178	2.07
37.5	1.771	2.03	37.5	0.186	2.08
40	1.845	2.05	40	0.194	2.09
45	1.997	2.07	45	0.210	2.11
50	2.151	2.09	50	0.226	2.13

** Charts developed by interpolating data from *International Critical Tables*, Vol. 6, pp. 229-253, McGraw-Hill Book Co., NY.

B. Sodium Chloride* (NaCl)

Saturated solutions at all temperatures		
°C	mS/cm	%/°C (to 25°C)
0	134.50	1.86
5	155.55	1.91
10	177.90	1.95
15	201.40	1.99
20	225.92	2.02
25	251.30	2.05
30	277.40	2.08

Concentration: 0.5 mole/liter		
°C	mS/cm	%/°C (to 25°C)
0	25.90	1.78
5	29.64	1.82
10	33.61	1.86
15	37.79	1.90
20	42.14	1.93
25	46.65	1.96
30	51.28	1.99
35	56.01	2.01
37.5	58.40	2.02
40	60.81	2.02
45	65.65	2.04
50	70.50	2.05

Concentration: 1 x 10^{-1} mole/liter		
°C	mS/cm	%/°C (to 25°C)
0	5.77	1.83
5	6.65	1.88
10	7.58	1.92
15	8.57	1.96
20	9.60	1.99
25	10.66	2.02
30	11.75	2.04
35	12.86	2.06
37.5	13.42	2.07
40	13.99	2.08
45	15.14	2.10
50	16.30	2.12

Concentration: 1 x 10^{-2} mole/liter		
°C	mS/cm	%/°C (to 25°C)
0	0.632	1.87
5	0.731	1.92
10	0.836	1.97
15	0.948	2.01
20	1.064	2.05
25	1.186	2.09
30	1.312	2.12
35	1.442	2.16
37.5	1.508	2.17
40	1.575	2.19
45	1.711	2.21
50	1.850	2.24

Concentration: 1 x 10^{-3} mole/liter		
°C	mS/cm	%/°C (to 25°C)
0	0.066	1.88
5	0.076	1.93
10	0.087	1.98
15	0.099	2.02
20	0.111	2.07
25	0.124	2.11
30	0.137	2.15
35	0.151	2.19
37.5	0.158	2.20
40	0.165	2.22
45	0.180	2.25
50	0.195	2.29

* Charts developed by interpolating data from the *CRC Handbook of Chemistry and Physics*, 42nd ed., p. 2606, The Chemical Rubber Company, Cleveland.

C. Lithium Chloride* (LiCl)

Concentration: 1 mole/liter			Concentration: 1×10^{-1} mole/liter		
°C	mS/cm	%/°C (to 25°C)	°C	mS/cm	%/°C (to 25°C)
0	39.85	1.82	0	5.07	1.87
5	46.01	1.85	5	5.98	1.85
10	52.42	1.89	10	6.87	1.85
15	59.07	1.92	15	7.75	1.85
20	65.97	1.95	20	8.62	1.85
25	73.10	1.98	25	9.50	1.86
30	80.47	2.02	30	10.40	1.88
35	88.08	2.05	35	11.31	1.91
37.5	91.97	2.07	37.5	11.78	1.92
40	95.92	2.08	40	12.26	1.94
45	103.99	2.11	45	13.26	1.98
50	112.30	2.15	50	14.30	2.02

Concentration: 1×10^{-2} mole/liter			Concentration: 1×10^{-3} mole/liter		
°C	mS/cm	%/°C (to 25°C)	°C	mS/cm	%/°C (to 25°C)
0	0.567	1.88	0	0.059	1.93
5	0.659	1.92	5	0.068	2.03
10	0.755	1.96	10	0.078	2.12
15	0.856	2.00	15	0.089	2.19
20	0.961	2.04	20	0.101	2.25
25	1.070	2.08	25	0.114	2.28
30	1.183	2.12	30	0.127	2.31
35	1.301	2.16	35	0.140	2.32
37.5	1.362	2.18	37.5	0.147	2.32
40	1.423	2.20	40	0.154	2.31
45	1.549	2.24	45	0.166	2.29
50	1.680	2.28	50	0.178	2.25

D. Potassium Nitrate** (KNO_3)

Concentration: 1×10^{-1} mole/liter			Concentration: 1×10^{-2} mole/liter		
°C	mS/cm	%/°C (to 25°C)	°C	mS/cm	%/°C (to 25°C)
0	6.68	1.78	0	0.756	1.77
5	7.71	1.79	5	0.868	1.80
10	8.75	1.81	10	0.984	1.83
15	9.81	1.83	15	1.105	1.86
20	10.90	1.85	20	1.229	1.88
25	12.01	1.87	25	1.357	1.90
30	13.15	1.90	30	1.488	1.93
35	14.32	1.92	35	1.622	1.95
37.5	14.92	1.94	37.5	1.690	1.96
40	15.52	1.95	40	1.759	1.97
45	16.75	1.97	45	1.898	1.99
50	18.00	2.00	50	2.040	2.01

* Charts developed by interpolating data from the *CRC Handbook of Chemistry and Physics*, 42nd ed., p. 2606, The Chemical Rubber Company, Cleveland.
** Charts developed by interpolating data from *International Critical Tables*, Vol. 6, pp. 229-253, McGraw-Hill Book Co., NY.

E. Ammonium Chloride* (NH_4Cl)

Concentration: 1 mole/liter			Concentration: 1×10^{-1} mole/liter		
°C	mS/cm	%/°C (to 25°C)	°C	mS/cm	%/°C (to 25°C)
0	64.10	1.60	0	6.96	1.82
5	74.36	1.53	5	7.98	1.88
10	83.77	1.45	10	9.09	1.93
15	92.35	1.37	15	10.27	1.97
20	100.10	1.29	20	11.50	2.00
25	107.00	1.21	25	12.78	2.03
			30	14.09	2.06
			35	15.43	2.07
			37.5	16.10	2.08
			40	16.78	2.08
			45	18.12	2.09
			50	19.45	2.09

Concentration: 1×10^{-2} mole/liter			Concentration: 1×10^{-3} mole/liter		
°C	mS/cm	%/°C (to 25°C)	°C	mS/cm	%/°C (to 25°C)
0	0.764	1.84	0	0.078	1.88
5	0.889	1.86	5	0.092	1.90
10	1.015	1.88	10	0.105	1.91
15	1.144	1.91	15	0.119	1.93
20	1.277	1.94	20	0.133	1.95
25	1.414	1.97	25	0.148	1.98
30	1.557	2.02	30	0.162	2.01
35	1.706	2.06	35	0.178	2.04
37.5	1.782	2.08	37.5	0.186	2.06
40	1.860	2.10	40	0.194	2.07
45	2.020	2.14	45	0.210	2.11
50	2.186	2.18	50	0.227	2.15

* Charts developed by interpolating data from the *CRC Handbook of Chemistry and Physics*, 42nd ed., p. 2606, The Chemical Rubber Company, Cleveland.

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